Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2010 - 12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814 935 1081

5. **Grant SAP Number:** 4100050904

6. **Project Number and Title of Research Project:** 23. Developing an Animal Model of Cognitive Bias to Study the Impact of Emotion on Health and Behavior

7. **Start and End Date of Research Project:** 9/1/2010 - 12/31/2013

8. **Name of Principal Investigator for the Research Project:** Victoria Braithwaite, PhD

9. **Research Project Expenses**.

   9(A) Please provide the amount of health research grant funds spent on this project for the entire duration of the grant, including any interest earned that was spent:

   $ 277,046

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Braithwaite	Principle Investigator	10% Yr 1, 25% Yr 2-3	$26,451.00
Chaby	Research Associate	100% Yr1-3	21,452.00
Neuberger	Assistant Professor	30% Yr 3	24,772.50
Vasey	Research Associate	30% Yr 2-3	20,220.00
White	Undergraduate Assistant	10% Yr 2	725.00
Wang	Undergraduate Assistant	15% Yr 1-2	3,443.75
Hirrlinger	Undergraduate Assistant	15% Yr 2-3	2,112.00

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Braithwaite	Professor	60% Yr 1-3
Cavigelli	Associate Professor	30% Yr 1-3
Grigson	Professor	10% Yr 1

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes___X____ No_____

If yes, please indicate the source and amount of other funds:

Penn State start-up funds awarded to Braithwaite were used to cover the cost of summer assistant help and to purchase a new laptop needed for behavioral observations, as well as smaller items of equipment for behavior trials and reagents for some of the brain preservation work ($8,250).

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes___X____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Investigating sensitive periods for effective exposure to environmental enrichment	☐NIH ☐ Other federal (specify:_____) X Nonfederal source (specify: Swedish Research Council)	Oct 2012	$225, 800	$ 225,800
Stress as a signal to prepare for future adversity: The effects of exposure to predation threat on behavior and cognition under future threat	☐NIH X Other federal (specify:_NSF_) ☐ Nonfederal source (specify:_)	Oct 2013	$19,434	$ Pending
The physiology and development of temperament	☐NIH X Other federal (specify:_NSF_) ☐ Nonfederal source (specify:_)	Jan 2014	$ Pre-proposal	$ Pending

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___X_____ No_____

If yes, please describe your plans:

Dr Cavigelli and I will also apply for an R21 for NIMH to be submitted in June 2014.

12. Future of Research Project. What are the future plans for this research project?

We intend to further investigate the long-term behavioral consequences of experiencing chronic, unpredictable social and physical stressors during adolescence. We are particularly interested in determining the impact that these experiences have on the behavioral coping and resilience capacities of adults. We also plan to examine the neuroendocrine mechanisms underpinning these effects on behavior by looking for long term changes in glucocorticoid receptor (both MR and GRs) expression and responsivity in the brain, and by looking at the connectivity between the forebrain and the amygdala.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female	12		1	1
Unknown				
Total	**12**		**1**	**1**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown	12		1	1
Total	**12**		**1**	**1**

	Undergraduate	Masters	Pre-doc	Post-doc
White	11		1	1
Black	1			
Asian				
Other				
Unknown				
Total	**12**		**1**	**1**

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____X___

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes___X____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

The project established a new, successful collaboration between Cavigelli and Braithwaite. They now have two joint grant proposals in preparation for submission to NIMH and NSF, and have a joint publication, a manuscript in review and three other manuscripts in preparation.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes____X____ No_____

If yes, please describe the collaborations:

The drug addiction work was undertaken at Hershey Medical School with Dr. Sue Grigson.

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes__X_____ No_____

If yes, please describe involvement with community groups that resulted from the research project:

The RA and now pre-doctoral graduate student, Lauren Chaby, used the ideas generated in this research project to prepare materials about brain and behavior for a site visit to Miles Township Elementary in Rebersburg PA where she was able to bring science about the brain into the classroom.

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

The aim of this research project was to create a superior animal model of emotion. Using novel testing paradigms that directly measured rodent cognitive appraisal strategies, we intended to create a new animal model of emotional states. Our goal was to quantify emotional state by evaluating behavioral, physiological and cognitive biases. The work had two main objectives.

Objective (i) To demonstrate that cognitive biases in rats, which we argue are analogous to human optimism or pessimism, are associated with neuroendocrine and behavioral biases that accompany such traits in humans. By doing so, our aim was to develop a more comprehensive rodent model of emotion that includes assessment of cognitive as well as physiological and behavioral biases.

Objective (ii) To develop a rearing paradigm to create different populations of rats with contrasting long-term cognitive biases (i.e. groups of animals with either more positive or more negative long-term affective states). These rats will be used to determine health consequences of cognitive bias. Specific to this project we planned investigate choice behaviors related to drugs of abuse, but in the future these animals could be used to investigate how emotional state affects resilience and plasticity in response to different kinds of challenge such as compromised health, stress and pain.

Progress made:
Objective 1:
Good progress was made to achieve this objective. In this phase of our study, we wanted to compare a novel measure of affective state in rats with conventional measures of affective-state-related behavior and physiology to determine if the novel measure is related to the conventional measures. The affective state measure that we used had previously been proposed as a method to assess cognitive bias in animals. The test is based around the idea that animals taught to discriminate between two stimuli (e.g. a rewarded white food bowl and an unrewarded black food bowl) will vary in their categorization of ambiguous stimuli (e.g. a grey food bowl) that is intermediate between the two alternative training stimuli. Animals with a more positive affective state are expected to categorize the grey bowl as through it is rewarded (and more like the white bowl stimulus – a positive bias), whereas negative affective state is likely to induce a conservative outlook and the animal here categorizes the grey food bowl as closer to black and so unrewarded (a more negative bias).

We began by working with a hooded strain of rat (Long Evans) as previously published work had been performed with Lister Hooded rats (Burman et al. 2008a,b). We tried 3 different experimental paradigms that required rats to learn positive and negative associations and then respond to probe tests with ambiguous cues, but found it difficult to get consistent responses from the Long Evans rats. We therefore switched rat strain to Sprague Dawley rats, these animals were much more amendable to training, they provided more consistent data and so this strain was used for all remaining experiments.

As the cognitive bias paradigms required lengthy training (i.e. to learn discriminations

between the different stimuli before probe trials could be tested), we decided to include an additional assay to look at a possible effect of anhedonia associated with removal of enrichment, while not quite the same as cognitive bias, a measure of anhedonia is still considered to be a measure of affective state. For this we compared the sucrose intake of rats that were housed in standard conditions with enrichment to rats that had lost their cage enrichment 1 month earlier.

Using this technique we were able to show a clear effect of removing enrichment; animals that lost enrichment had a significantly lower intake of sucrose solution indicating that enrichment loss induced a state of anhedonia in these animals (Fig. 1). Interestingly, this effect was observed even though the rats were tested 1 month after the rats had lost their cage enrichment suggesting a long lasting effect of enrichment loss on affective state. This result has been written up and is currently in review at *Applied Animal Behaviour Science* (Chaby, Cavigelli, Hirrlinger & Braithwaite: Removing enrichment alters coping but returning enrichment promotes recovery).

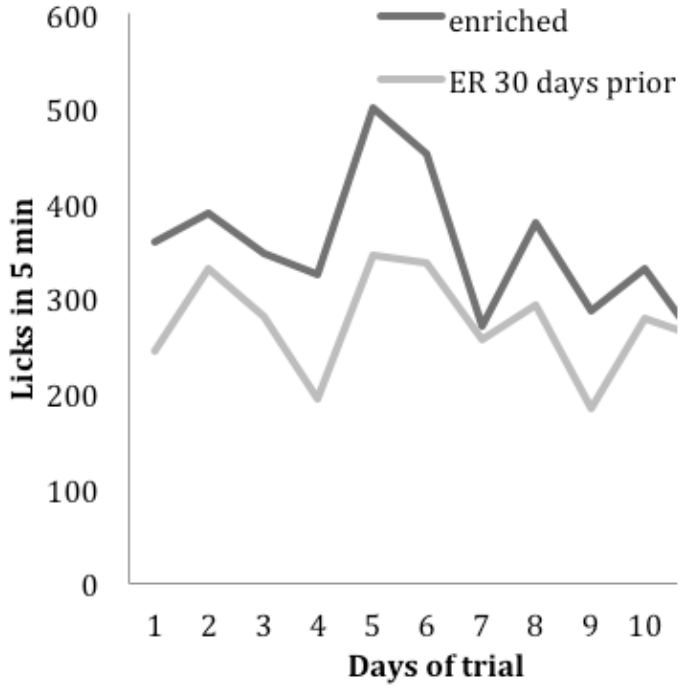

Fig. 1 Sucrose intake to assess long term effects of enrichment removal. Enrichment removal (ER) negatively impacts sucrose intake – as the pre-shift licking rate is lower in enrichment loss (ER) group before the downshift after day 12 ($F_{1,10} = 6.52$, $p = 0.03$).

One month after the enrichment loss, blood was collected from the rats to quantify plasma corticosteroid levels, however no differences were found in the circulating levels of corticosteroid ($T_{1,10} = 0.9$, $p = 0.37$). This lack of difference may be because any physiological response to changes such as the loss of enrichment may be short-lived. Thus, blood samples taken over a month after a change to the environment may have had time to adjust to pre-manipulation levels. This likely plasticity of the stress response would be an interesting result to follow up in future work. It would, for example, be interesting to compare the plasma corticosteroid levels within a few days

of enrichment loss (we have collected blood samples to permit this and an undergraduate assistant is currently working on these for her Honors project). Similarly, it could be helpful to look at the corticosteroid receptors in the brain, rather than circulating levels of coritcosteroid in blood plasma. This could be done by *in situ* hybridization techniques on sections of the brain to quantify glucocorticoid (GR) and mineralcorticoid (MR) receptor number and distribution in the forebrain (We have preserved brains from stressed and non-stressed rats and these will be sectioned and the stress receptors will be compared by Lauren Chaby as part of her PhD research project). These may show differences, whereas the circulating levels of corticosteroid may more readily be adjusted, and more plastic.

Data collected from standard anxiety assays provided a behavioral measure. Here, we found that anxiety levels remained consistent across trials and there was no overall effect of enrichment loss on rat anxiety (open field trials (see Fig. 2) and novel object tests; $F_{1,6} = 0.24$, $p=0.63$). Thus anxiety levels were not demonstrably perturbed by enrichment loss.

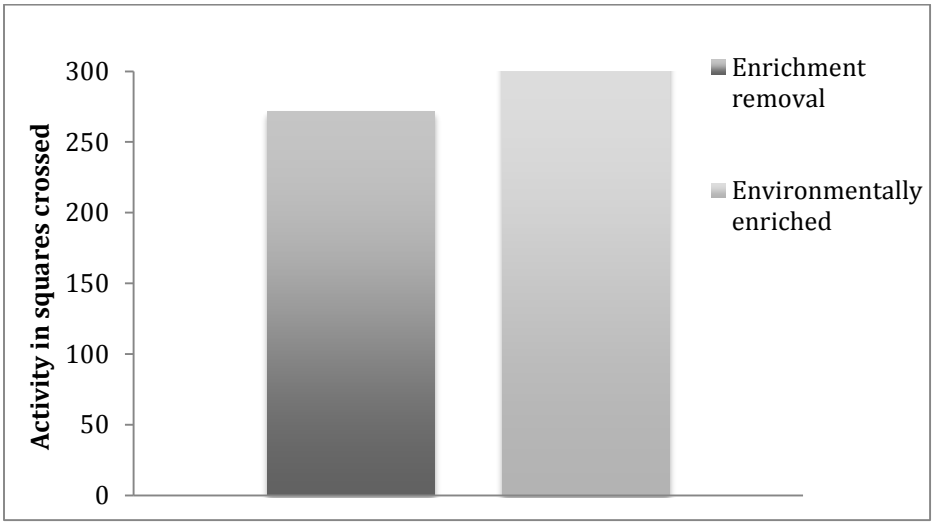

Fig. 2: Activity in open field arena in rats that experienced standard enriched housing, or that lost their enrichment prior to testing ($F_{1,6} = 0.42$; $p = 0.53$).

When the different trials of objective 1 were compared, we were not able to find consistent correlations between affective state, behavior and physiology, but further tests are needed to address the corticosteroid measures closer to the time of enrichment loss.

What the experiments in this objective have shown is that rats that have experienced the loss of their cage enrichment do express a negative affective state in the form of anhedonia. This result adds to the growing literature on the effects and value of enrichment, and on assays of affective state in rodent models (Harding et al. 2004; Burman et al. 2008a,b; Brydges et al. 2011; Brydges et al. 2012).

Objective 2:
Our goal for this objective was to develop a reliable rearing paradigm that would establish contrasting long-term cognitive biases in rats (i.e. generate groups of animals with either more positive or more negative long-term affective states). This phase of the research has worked extremely well, and produced multiple, compelling lines of evidence that applying chronic, unpredictable stressors during adolescence sets up long term changes in affective state (Chaby et al. 2013). The effects of exposure to Chronic Mild Stress (CMS) during adolescence were studied in 16 rats. Animals were split into 2 groups; 8 that were maintained standard housing and acted as controls, and 8 that were exposed to CMS during adolescence. For the CMS group, unpredictable stressors were presented daily for 40 days (day 30-70) excluding 8 days of rest that were spread intermittently across this time period (see the timeline in Fig. 3). To maximize unpredictability, physical and social stressors were presented randomly across the 12:12 reversed L:D cycle, with an average of 3 physical and social stressors between each rest day (see Table 1).

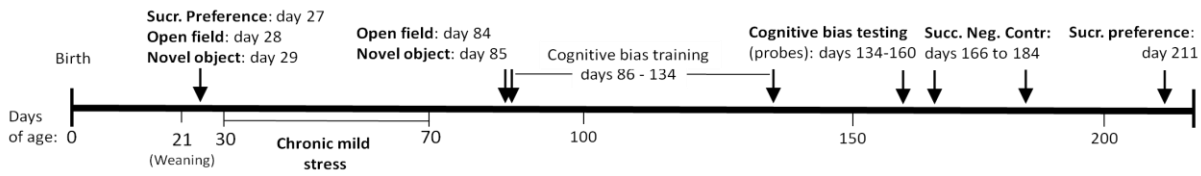

Fig. 3: Timeline of adolescent stress and assays in adulthood used for Objective 2a (cognitive bias, successive negative contrast, sucrose preference, and exploratory behavior).

Table 1: Summary of the kinds of unpredictable stressor experienced by half the rats.

Physical	
Smaller cage	Rat pairs were housed for 4 hours in a cage with a 25% reduction in volume from the 25 x 46 x 20 cm^3 standard home cage.
Damp bedding	While rats were temporarily in a transfer cage, 200ml of water was mixed into 2/3 of the bedding of the home cage. After 6 hours in the damp bedding, pairs were transferred to a clean home cage.
Cage tilt	Home cages were tilted at a 30° angle for 6 hours.
Social	
Isolation	Rats were housed individually for 1.5 hours in a clean home cage with a 3” diameter PVC tube and 2.5 x 2.5 x 8 cm^3 pine block.
Crowding	Sets of 2 rat pairs were aggregated into one clean, unmarked cage for 4 hours; iterations of pair combinations were balanced.
Foreign bedding	Experimental pairs were housed in the empty home cage of a pair of older conspecifics for 12 hours.

Objective 2a: Assessing the consequence of Chronic Mild Stress during adolescence on long term affective state in adulthood

A cognitive bias task using a paradigm similar to Brydges et al. (2011) was used to assay stimulus interpretation in stressed and control rats. Animals were trained to associate a conditioned stimulus (sandpaper of a rough or a fine grade) with a bowl location and color containing an available food reward. To do this, individuals were placed in a 40 x 45 cm opaque plastic start box, connected to a goal box by an 80cm PVC pipe lined with sandpaper (see Fig. 4). The goal box contained a white bowl and a black bowl separated by a partition, which ensured that animals had to choose a bowl upon exiting the PVC tunnel (bowl location, color and reward status were counterbalanced across both control and treatment groups). Of the two available bowls, one was associated with a high-value reward (3 Cheerios), the other with a low-value reward (1 Cheerio). To balance the scent

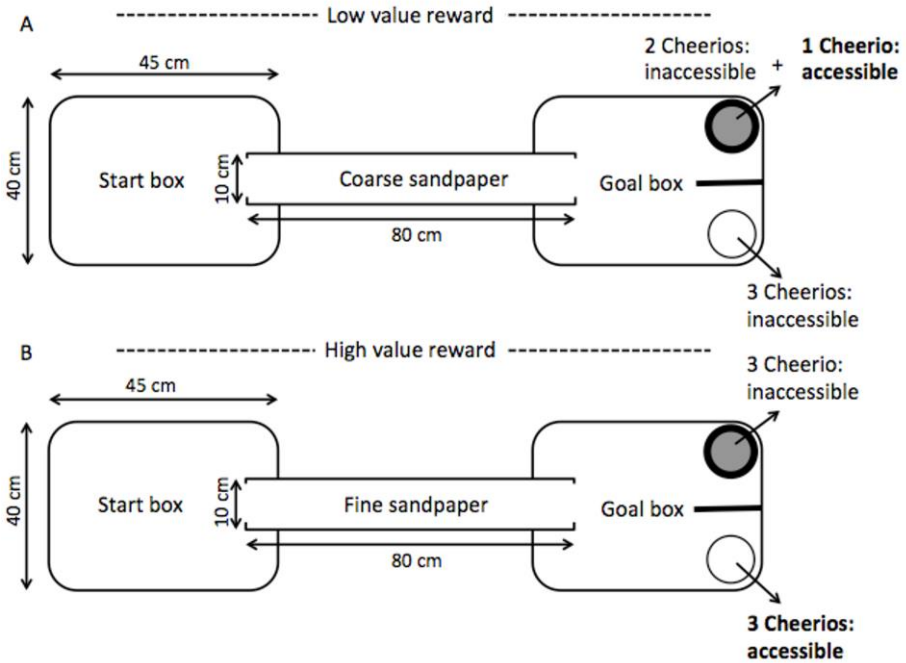

Fig: 4 Schematic diagram of cognitive bias testing chamber adapted from Brydges et al. (2011). Within a daily session, 2 trials of each example panel were presented. Panel A depicts a low-value reward trial; in this example, the coarse sandpaper provided a cue that the low-value (1 Cheerio) reward was accessible whereas, in Panel B, the fine sandpaper indicated a high-value reward (3 Cheerio) was present. After reaching a learning criterion, animals were given an ambiguous cue (sandpaper of intermediate grade). Interpretation of the ambiguous sandpaper was gauged by the choice of bowl that the rats approached during probe trials.

cues of the rewarded and unrewarded bowls each bowl always contained three Cheerios, but the accessibility of the rewards varied depending on the trial condition (See Fig. 4). A tactile cue lining the PVC tunnel and goal box indicated which bowl had an available

reward; one of two grades of waterproof sandpaper, coarse (P60) and fine (P1200), was paired with a specific reward type (e.g. coarse sandpaper signaled a low-reward in the black bowl on the left). Pairings of sandpaper grade, bowl color, bowl side, and reward value were counterbalanced. All elements of the testing chamber were cleaned with 70% ethanol between each trial.

To learn the stimulus-reward associations, rats were exposed to daily training sessions that consisted of 2 high-reward trials and 2 low-reward trials; the order of the 4 trials was randomized. Animals moved from the start box through the PVC tubing into the goal box, and chose either the "correct" rewarded or "incorrect" unrewarded bowl. A choice was defined as a rat either moving its nose or paw inside the bowl or touching the outside of the bowl.

A learning criterion was set at 3 out of 4 trials correct for 4 out of 5 days. The number of days to reach the learning criterion were evaluated to determine if adolescent stress impacts adult associative learning (animals stressed during adolescence showed no difference in the number of days to learn the associative task compared with controls; *t* test, stress: 26 $\pm$ 3 vs. control: 25 $\pm$ 5; $T_{1,10} = 0.419$, $P = 0.68$). After passing the criterion, 5 probe trials were conducted over 5 days where ambiguous/intermediate grade sandpaper was placed in the PVC tube connecting the start and goal boxes. On each day of probe testing 5 trials were run; in addition to the 4 standard trials, one probe trial using ambiguous sandpaper was randomly inserted into the normal sequence, but the last trial was never a probe trial. After choosing a bowl during the ambiguous probe trial, the bowl choice was noted as either a high or a low-reward categorization of the ambiguous mesh cue and the animal was allowed to consume the reward. Previous studies using unrewarded probe trials have found that animals learn that probes are never reinforced and stop responding during probe trials (Brilot et al., 2010; Doyle et al., 2010; Bateson and Matheson, 2007). To circumvent this, in the current study both high and low rewards were present during probe trials to avoid cessation of response.

All stressed animals interpreted the ambiguous cue as negative on the first day of probe testing, demonstrating a negative cognitive bias, that differed from the controls whose interpretations were half positive and half negative ($F_{1,8} = 5.000$, $P < 0.05$, $R^2 = 0.33$, Fig. 5). The difference in interpretation of the ambiguous cue between the control and adolescent-stressed animals was not evident during any of the following test days (RM ANOVA: $F_{1,14} = 1.073$, $P = 0.32$, Fig. 6).

Thus experiencing stress during adolescence has an effect on adult affective state, but this effect was only apparent in the first probe trial. We believe that this is because the rats are very quick to learn the meaning of the probe trials, thus the only truly reliable probe test is the first time that the animals encounter the intermediate grade of sandpaper on probe trial 1.

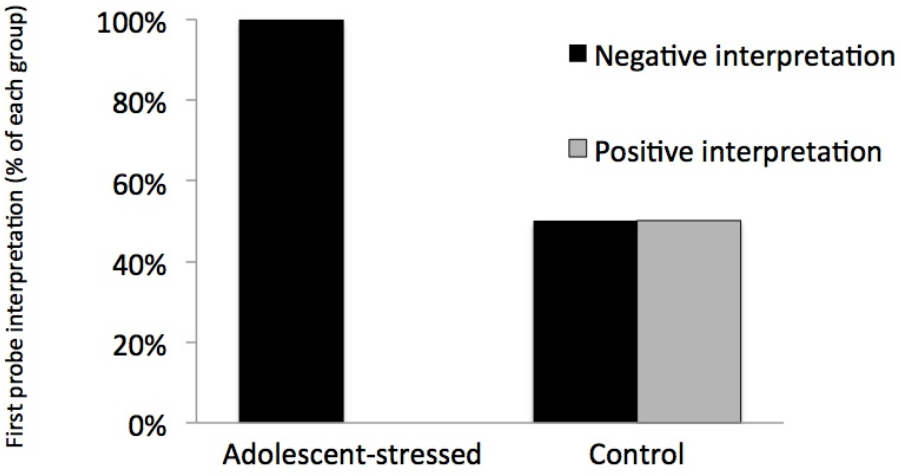

Fig. 5 Interpretation of a novel ambiguous stimulus on the first day of an ambiguous judgment test for cognitive bias. All animals that experienced stress during adolescence interpreted the ambiguous cue as negative, indicating a long-term negative cognitive bias, while control animals were equally likely to interpret the cue as positive or negative.

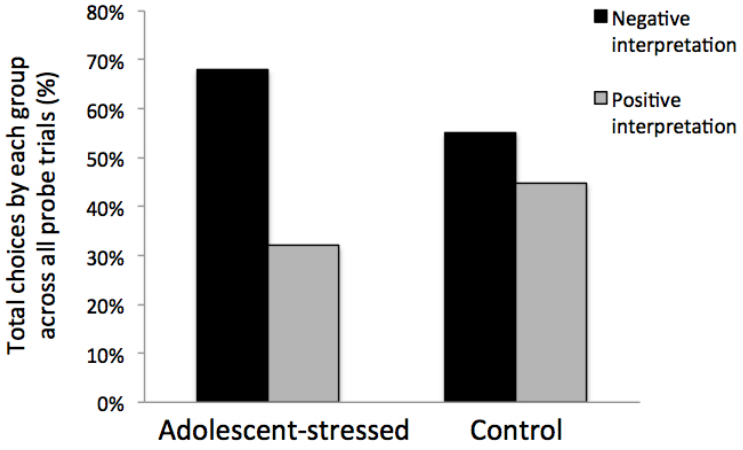

Fig 6: Interpretation of an ambiguous stimulus during all cognitive bias testing days. Rats that experienced stress during adolescence trended towards a negative cognitive bias across the 5 day testing period.

Alternative assays for affective state:
As the cognitive bias task was clearly only reliable for the first probe trial exposure, we decided to trial an additional task to quantify affective state; the Successive Negative Contrast (SNC) task that measures levels of frustration. This assay was attractive because it required less training, so we used this as an alternative measure of affective state (Mitchell & Flaherty 1998).

The SNC quantifies the response animals have to an unpredictable downshift in reward value. This task has previously been used to assess an animal's ability to cope with frustration, we therefore selected it to measure the potential for increased frustration in animals that had experienced a period of CMS as juveniles. The SNC assay we adopted measured the response rats had to an unpredicted decrease in sucrose reward (a drop from 32% to 4% sucrose solution) and we compared this response in rats maintained in standard housing to rats that received CMS during their adolescent stage. To ensure reward salience, 2 hours of food deprivation preceded each trial. The high value reward, 32% sucrose solution, was presented for 12 days; on the 13th day of testing, the reward was downshifted to a 4% sucrose solution and administered for 7 days. Motivation to consume sucrose solution was quantified with a device attached to a counter that registered each lick through the closing of a circuit and recorded licking rates on the spout of the sucrose solution bottle over a 5-minute period.

All 16 animals were exposed to a 12-day training phase where they had access to 32% sucrose solution, and then a drastic downshift on day 13 to a 4% sucrose solution, administered for 7 days. Motivation to consume the sucrose solution was quantified over a 5-minute period.

Response to reward down-shift was greater in the adolescent-stress group than the controls (Paired *t* test, stress vs. control group licks: 26 $\pm$ 42 vs. 85 $\pm$ 70; $T_{1,14} = -2.22$ $p = 0.04$, Fig. 7). No differences were found in pre-shift lick rates between groups (RMANOVA, stress vs. control group licks: 182 $\pm$ 95 vs. 178 $\pm$ 101; $F_{1,6} = 0.09$, $p = 0.77$) or over time ($F_{1,6} = 4.02$, $p = 0.37$), nor was there an interaction ($F_{1,6} = 1.173$, $p = 0.61$). Post-shift rates changed over time as rats returned to pre-shift licking rates (RMANOVA, $F_{1,6} = 9.91$, $p = 0.01$), but stress and control animals did not differ in their post-shift lick rates (RMANOVA, stress vs. control group licks: = 165 $\pm$ 125 vs. 176 $\pm$ 111; $F_{1,6} = 0.003$, $p = 0.95$), nor was there an interaction effect ($F_{1,6} = 0.64$, $p = 0.70$).

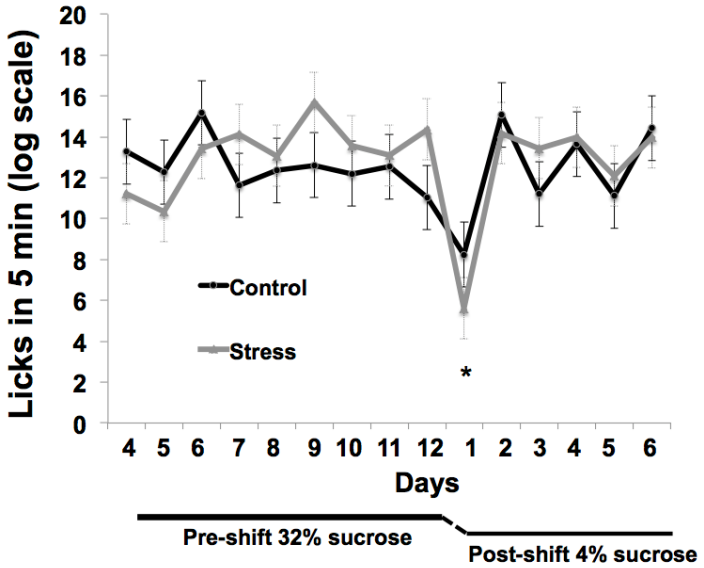

Fig. 7: Animals that experienced adolescent chronic mild stress exhibit increased sensitivity to devaluation of a reward as measured by successive negative contrast (SNC).

These measures of affective state were then related to other behavioral indicators of anxiety. To compare activity levels between adolescent-stress and control animals, activity in an open field arena (48"x 48") was quantified at two time points (28 and 84 days of age, pre and post chronic mild stress, see Fig. 3). During video analysis an 8 x 8 grid was used to quantify activity by counting the number of squares crossed on the grid. Crossing of grid squares along the walls and the proportion of time spent in squares along the walls of the arena were quantified as indicators of thigmotaxis, a positive correlate of anxiety. The day following open field assessment, response to novelty was quantified by measuring time to leave the home base and latencies to approach two novel objects in the arena (at 29 and 85 days of age). The novel objects varied in texture, color, and size. Each object was unique to the iteration of the task.

Open field activity scores

Activity, as measured by squares crossed, increased over time (RMANOVA effect of time: $F_{1,7} = 8.45, p = 0.01$). There was no effect of stress treatment (RMANOVA effect of stress: $F_{1,7} = 0.09$, $p = 0.76$), nor was there an interaction between stress condition and time (RMANOVA stress x time interaction: $F_{1,7} = 1.42$, $p = 0.25$ main effect. No difference in activity was detected in number of squares crossed at time point 1 or 2 (Paired *t* tests, stress vs. control group: T1: 244 $\pm$ 30 vs. 233 $\pm$ 86; $T_{1,14} = 0.70$, $p = 0.49$; T2: 282 $\pm$ 29 vs. 303 $\pm$ 40; $T_{1,14} = 1.11$, $p = 0.28$).

Thigmotaxis decreased over time, as measured by time at edge (RMANOVA effect of time: $F_{1,7} = 97.685$, $p < 0.001$). There was no effect of stress condition (RMANOVA effect of stress: $F_{1,7} = 0.11$, $p = 0.74$), and no interaction between stress and change over time (RMANOVA stress x time interaction: $F_{1,7} = 0.36$, $p = 0.56$). No difference in activity was detected in number of squares along the edge crossed at time point 1 or 2 (Paired *t* tests, stress vs. control group: T1: 283 $\pm$ 12(s) vs. 282 $\pm$ 10(s); $T_{1,14} = 0.16$, $p = 0.88$; T2: 233 $\pm$ 15(s) vs. 238 $\pm$ 21(s); $T_{1,14} = 0.50$, $p = 0.62$).

Novel object
Latency to leave "home base" decreased over time (RMANOVA effect of time: $F_{1,7}$ = 11.18, p = 0.005). There was no main effect of treatment (RMANOVA effect of stress: $F_{1,7}$ = 1.28, p= 0.28), but there was a significant interaction between treatment and time across the two tests (RMANOVA stress x time interaction: $F_{1,7}$ = 8.70, p = 0.01). During the novel object test 15 days after the completion of the chronic mild stress treatment (day 85), rats exposed to adolescent stress left the home base PVC shelter faster than control animals (Paired *t* test, stress vs. control group exit latency: 2.3 $\pm$ 0.6 vs. 7.3 $\pm$ 1.8; $T_{1,14}$ = -2.24, p = 0.04) whereas there was no baseline difference in latency to leave the home base prior to stress exposure during time point 1 (day 29, see Figure 1) (Paired *t* test, stress vs. control group exit latency: 2 $\pm$ 0.5 vs. 1.7 $\pm$ 1; $T_{1,14}$ = 0.34, p = 0.74).

Impulsivity
To determine whether the rats varied in their decision making ability we compared the speed with which the animals would correct a wrong choice. This was measured by comparing the speed with which rats changed from a feeder with an inaccessible food reward to start feeding at a feeder where they could obtain the reward. Animals that had been stressed during adolescence were faster at correcting wrong decisions and abandoned the unrewarded bowl faster than control rats (Paired *t* test: $T_{1,14}$ = -3.24, p = 0.006, Fig. 8).

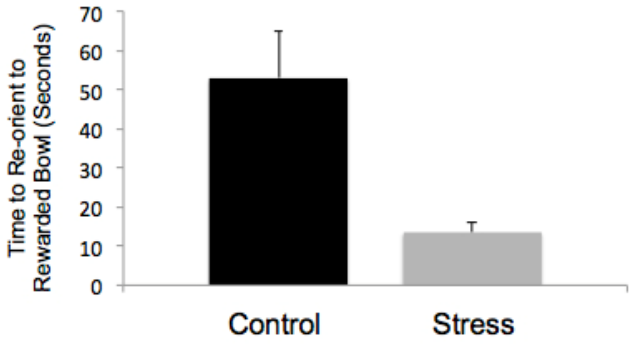

Fig. 8: Latency to switch to the correct bowl after first incorrect choice.

Corticosterone challenge
Following a baseline blood collection for basal corticosterone, animals were individually transferred to a cage 75% smaller than the home cage, 18 x 29 x 13 cm^3, and devoid of enrichment, in order to stimulate a stress response. Peak corticosterone was evaluated with a second blood sample collected at 30 min. Following the peak collection, animals were returned to pair-housed home cages. A third recovery sample was collected 80 min after baseline collection to assess clearance of stress hormone. Plasma corticosterone was assessed with a radioimmunoassay (MP Biomedicals, Solon, OH) and levels radioactively bound CORT was measured with a Gamma Counter.

In comparison with control rats, the baseline and stress induced corticosterone in animals exposed to CMS tended to have a lower level of plasma corticosterone before, during and

after exposure to an acute stressor, but this was not significant ($F_{1,9} = 3.45$; $p = 0.09$, Fig. 9). We believe that with a larger sample size this effect would have been significant, however, by the time this assay was run we had lost 2 animals in each group (reducing n to 6). (We collected plasma from stressed and control animals in which ACTH and DEX were used to generate a stress challenge to quantify the reactive scope of the animals. These samples are currently being processed as part of an undergraduate student Honors thesis due to be completed in April 2014).

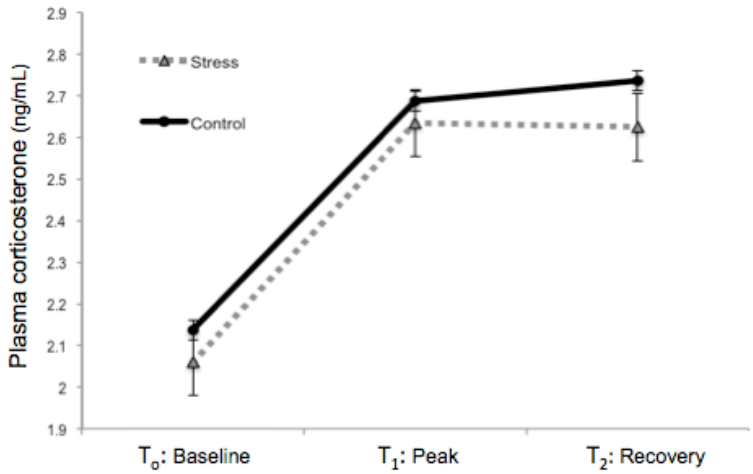

Fig. 9: Comparison of plasma corticosterone before, during and after experiencing an acute stressor for rats reared in standard conditions or exposed to Chronic Mild Stress during adolescence.

Adrenal weights

In the absence of HPA axis activation the adrenal gland can atrophy. To test for this at the end of the experiment the rats were perfused with formaldehyde, left and right adrenal glands were isolated; surrounding fat was removed and weights were obtained. Preceding adrenal collection, whole body weights were obtained to allow a calculation of adrenal weight relative to total body weight. As stress can also impact somatic lateralization, adrenal gland within individuals was also considered.

There were no observable effects on adrenal weight within or between animals. After standardizing adrenal weights for body weight, adrenal weight/total body weight, t-tests compared left stressed adrenals, $6.87_E10^{-5} \pm 0.79_E10^{-5}$(g), to left control adrenals $7.41_E10^{-5} \pm 1.10_E10^{-5}$(g) and right stressed adrenals, $6.76_E10^{-5} \pm 1.57_E10^{-5}$(g), to right control adrenals $6.50_E10^{-5} \pm 1.00_E10^{-5}$(g) ($F_{1,9} = 1.29$, $p = 0.29$, $F_{1,9} = 1.63$, $p = 0.24$, respectively). Lateralization in adrenal gland within individual was assessed with a repeated measures ANOVA ($F_{1,14} = 0.14$, $p = 0.72$).

Of the many results obtained in Objective 2b, we believe the most exciting observations are that exposure to chronic, unpredictable stress during adolescence established long term changes in susceptibility to frustration and an increase in impulsivity. These changes are apparent even though the last experience of CMS was 2-3 months prior to the assays of frustration and impulsivity. This key observation indicates that unpredictable stress

during adolescence can induce significant long term behavioral deficits. While previous work has explored the neuroendocrine effects of CMS during adolescence (McCormick et al. 2008, 2012), to our knowledge, our results are the first to show these long term cognitive and behavioral effects. We are currently following these observations up with current experiments and we are preparing an R21 for NIMH to further investigate these effects and to determine the mechanisms underpinning them (for June 2014).

Objective 2b: Assessing the consequence of Chronic Mild Stress during adolescence on exposure to drugs of abuse as adults

This phase of the study was conducted in year 1 when we were still using Long Evans hooded rats. Eleven rats from Objective 2a were transported to Hershey Medical School (The sample size was reduced as some animals were lost to illness, or needed to be sacrificed as sentinels for disease before the animals could change labs).

Over 2 days, the rats (CMS n=6 and Control n=5) were implanted with jugular catheters in the Grigson Lab at Hershey. Following 6 days of recovery, animals were placed on a water restriction phase but were given access to a weak saccharin solution while in self-administration chambers to habituate the animals to the chambers and to encourage the animals to lick on the spouts. During 3 days of habituation, a saccharin spout was placed on one of three different spout locations each day, left, center, then right. After habituation, animals were returned to *ad-lib* water access on the third afternoon. The next day the animals were placed in chambers for 7 successive days of self-administration of 0.33mg cocaine per infusion on a fixed ratio of 10 active spout responses per infusion (FR10). Saccharin was available on the left spout for 5 min, and then retracted. At this time the center (inactive) and right (active) spouts became available for response, with the right, active spout having a cue light above it. This regimen normally works well with Sprague-Dawley rats, but for some reason was not found to produce drug-seeking behavior in the Long Evans rats. So this was followed by 4 days of FR20, then a single day of progressive ratio during which each successive infusion required 50 additional spout responses than the previous infusion (10, 60, 110, 160, etc.). This was followed by a day of extinction/reinstatement. The animals were placed in self-administration chambers for 5-min access to saccharin, then 120 minutes access to center and right spout, but with no cocaine infusions. After 120 minutes of extinction, a single 0.33 mg infusion of cocaine was given as a reinstatement stimulus. This was followed by another 120 minutes of center and right spout access with no infusions.

Interestingly, 7 days of drug taking on the FR10 schedule was not enough to split the groups into either low- and high-drug-seekers or low- and high-drug-takers based on spout responses or total infusions, respectively. An additional four days of FR20 did not present enough of a challenge either. To further test the drug-taking behavior of the subjects, the progressive ratio schedule was introduced on the 12th day, however there was no significant difference in spout responses or infusions taken. Due to time limitations, a short extinction/reinstatement experiment was attempted to test the propensity of each group to continue to work for unavailable drug and to relapse into

drug-taking.

The rats exposed during adolescence to CMS tended to make fewer total active spout responses than the control rats during extinction/reinstatement, however the difference was not significant (T test, p=0.09), which could be a result of low statistical power. Conversely, the control group tended to make fewer spout responses on the inactive spout. Again, this difference was a trend, but did not reach the level of significance (p=0.06).

Consistent with spout responses, the total number of infusions tended to be lower for the stressed group. During the initial or extinction phase of the test, control subjects achieved significantly more infusions than did the stressed group (T test, p=0.01). While the control group tended to take more infusions during the reinstatement phase of the test, several measures failed to reach significance. Total post-priming infusions were not different (T test, p=0.35), latency to the first infusion post-priming was not different (T test, p=0.54), and number of infusions during the first 10 minutes post-prime did not reach significance (T test, p=0.56).

While these results were not predicted, they are interesting for two reasons. First they compliment other work we have done to address strain differences in affective state and between Sprague-Dawley (albino) and Long Evans (hooded) rats. The experiment from objective 2b, demonstrates that the Long Evans rats are more resilient to addiction than Sprague-Dawley rats (data from previous studies in the Grigson lab). Second, even though there is low statistical power (n=5 control, n=6 chromic mild stress rats), trends were observed in terms of tendency to seek out cocaine. The data suggest that exposure to chronic mild stress in adolescence may in fact decrease tendencies towards addiction.

Additional work undertaken through the project

One of the early observations from Objective 1 was that Long Evans rats were not ideal for the behavioral tasks we were training the animals to perform. This prompted us to obtain a cohort of Sprague Dawley animals for comparison. This part of the work raised unexpected and surprising findings associated with rat strain and we have now written this work up and plan to submit this to Behavioural Brain Research (Chaby et al. in review). In this comparison we explored the effects of enrichment loss on the two rat strains.

In summary, we found that enrichment removal had opposite effects on reward consumption in the two strains. Long-Evans animals that experienced enrichment removal increased their sucrose intake, while Sprague-Dawley rats with enrichment removal decreased their sucrose intake (RMANOVA effect of strain: $F_{1,11} = 4.82$, $p = 0.04$, Fig. 10). In the Successive Negative Contrast task, both strains responded to the unexpected downshift in reward by significantly dropping their response rate. Enrichment removal also had an effect here, increased successive negative contrast frustration in both strains. Coping response following sucrose devaluation was increased by enrichment removal and differed by strain (RMANOVA effect of strain: $F_{1,11} = 15.98$, $p < 0.01$; enrichment: $F_{1,11} = 6.65$, $p = 0.02$, Fig. 10).

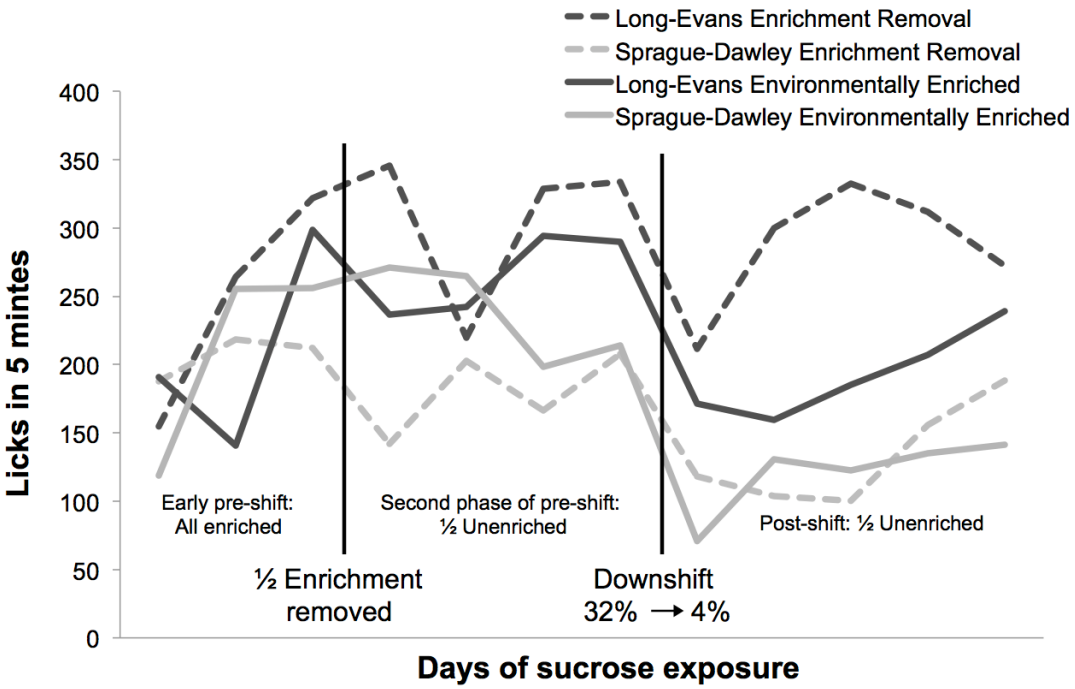

Figure 10: Successive negative contrast test results for enriched and enrichment-removed Long-Evans and Sprague-Dawley adult male rat strains. Across the entire test, number of licks changes with strain and across time (RMANOVA effect of strain: $F_{1,11} = 10.34$, $p = 0.01$; time: $F_{1,11} = 189.9$, $p < 0.01$). During the early pre-shift phase, wave and time impact lick number (RMANOVA effect of wave: $F_{1,11} = 13.49$, $p < 0.01$time: $F_{1,11} = 188.8$, $p < 0.01$). Response to the enrichment removal in the second phase of pre-shift differed by strain; Long-Evans rats that experienced enrichment removal increased their sucrose intake, while enrichment-removal Sprague-Dawley rats decreased their sucrose intake (RMANOVA effect of strain: $F_{1,11} = 4.82$, $p = 0.04$). Coping response in the post-shift phase was increased by enrichment removal and differed by strain (RMANOVA effect of strain: $F_{1,11} = 15.98$, $p < 0.01$; enrichment: $F_{1,11} = 6.65$, $p = 0.02$).

Successful Training Outcome:

Throughout this project Lauren Chaby, the graduate Research Assistant who was employed to assist with the rat experiments, has been given the opportunity to enroll on a PhD graduate program in Neuroscience at Penn State. Her early work on this project helped her establish her research interests and she is now well integrated into and excelling in her PhD research. We have also involved 12 undergraduate students who have either worked on independent study projects as part of this research program, or they have created thesis research projects for their Honors thesis.

Summary:
The results from the research project fulfilled almost all of the originally stated objectives, but additionally, we have found several novel and intriguing results that are leading to new research. We have shown a negative impact of cage enrichment loss on affective state, and that this effect is variable across rat strain. We have also shown that chronic exposure to unpredictable stress during a key phase of development, adolescence, has a long term negative impact on capacity to cope with frustration and it also increases impulsivity.

These results have led to two manuscripts for publication in peer reviewed journals (see #20 below for details), with three other manuscripts in preparation, we have obtained some additional funds for a Swedish post-doc to work on a follow-on project and we have two other grant applications submitted and pending. Finally, the long term behavioral effects of exposure to unpredictable stress during adolescence will form the basis of a R21 grant proposal for NIMH in June 2014.

References:
Chaby et al. (2013) *Frontiers in Neuroscience* 7, 328-335.
Brydges et al. (2011) *Animal Behaviour* 81, 169-175.
Brydges et al. (2012) *PLoS One* 7(10): e48143.
Burman et al. (2008a) *Animal Behaviour* 76: 801-809.
Burman et al. (2008b) *Biology Letters* 4: 330-333.
Harding et al. (2004) *Nature* 427: 312.
McCormick et al. (2008) *Behavioral Brain Research* 187, 228-238.
McCormick et al. (2012) *Hippocampus* 22, 1300–1312.
Mitchell & Flaherty (1998) *Physiology & Behavior 64*, 287-292.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?
_____Yes
_X___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?
_____Yes
__X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____ Yes
__X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. Long-term changes in cognitive bias and coping response as a result of chronic unpredictable stress during adolescence.	Chaby, L.E. Cavigelli, S.A. White, A. Wang, K. & Braithwaite, V.A.	*Frontiers in Human Neuroscience* 7, 328, 1-10.	April 2013	☐Submitted ☐Accepted ☒Published
2. Removing enrichment alters coping but returning enrichment promotes recovery	Chaby, L.E., Cavigelli, S. A., Hirrlinger, A. & Braithwaite, V. A.	*Applied Animal Behaviour Science*	January 2014	☒Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes__X_____ No_____

If yes, please describe your plans:

We have two other articles in preparation:

1) Chaby, L. E., Cavigelli, S. A. & Braithwaite, V. A. (in prep.) Anhedonic behaviour following the removal of environmental enrichment: Individual differences in coping with enrichment loss and successive negative contrast. *Behavioural Brain Research*

2) Chaby, L. E., Sheriff, M. J., Hirrlinger, A. M. & Braithwaite, V. A. (in prep.) Developmental stress improves future foraging under threat and alters decision making behavior. *Behavioural Brain Research*

3) Chaby, L. E., Cavigelli, S. A., Hirrlinger, A. Caruso, M. J. & Braithwaite, V. A. Chronic unpredictable stress during adolescence causes long-term anxiety. *Hormones and Behavior*

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The impact the project's results are in terms of a better understanding the long term impact of chronic unpredictable stress during adolescence. While this has been studied in terms of neuroendocrine changes, no previous work has specifically addressed the long term impacts on behavioral phenotype, yet we have found several. Although this work has been undertaken on a rodent model, we believe the results could have implications for human adolescents.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X___

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items
a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

NAME	POSITION TITLE
Braithwaite, Victoria Anne	Professor of Fisheries & Biology
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Oxford University, UK	BSc(Hons)	1988	Zoology
Oxford University, UK	PhD	1993	Animal Behavior
Glasgow University, UK	Postdoc	1993	Evolution & Behavior

B. Positions and Honors

Positions & Employment

1993 – 1994	Natural Environment Research Council, Postdoctoral Fellow, University of Glasgow, UK
1995 - 1999	Assistant Professor in Animal Behavior, The University of Edinburgh, UK
1999	Associate Professor in Animal Behavior, University of Edinburgh, UK
2006 – 2007	Sabbatical at Institute for Advanced Studies, Berlin, Germany
2007 -	Professor of Fisheries & Biology, Penn State University with full tenure
2008 -	Visiting Professor of Biology, The University of Bergen, Norway

Awards & Honors

1987	Kirkaldy Prize in Biological Sciences, Oxford University
1989-1992	Christopher Welch PhD Scholarship, Oxford University
1992	British Academic Research Collboration award
1995	Association for the Study of Animal Behavior Research Award
1999	Association for the Study of Animal Behavior Research Award
2005	Elected Fellow of the Royal Institute of Navigation
2006	Fisheries Society Medal
2011	Bellis Ecology Award

Federal Government public advisory committee

2006-7	UK Government Animal Procedures Advisory Panel

Other Experience and Professional Memberships

2011	Member of the International Society of Neuroethology
1988 -	Member, Association for the Study of Animal Behaviour
1989 -	Member, The Royal Institute of Navigtation
1992 -	Member, Fisheries Society
1996-1999	Consulting Editor, Animal Behaviour
2007 -	Member, American Fisheries Society

B. Selected Peer-reviewed Publications from last 3 years (from 89 publications)

Braithwaite, V. A. & Ebbesson, L. O. E. (2014) Pain and stress responses in farmed fish. OIE *Scientific and Technical Review*, Vol. 33 (1).

Salvanes, A. G. V., Moberg, O., Ebbesson, L. O. E., Nilsen, T.O., Jensen, K. H. & Braithwaite, V. A. (2013) Environmental enrichment promotes neural plasticity and cognitive behaviour in fish. *Proceedings of the Royal Society Series B.* 280.

Grassie, C., Braithwaite, V. A., Nilsson, J., Nilsen, T. O., Teien, H-C., Handeland, S. O., Stefansson, S. O., Tronci, V. M. Gorissen, G. Flik & Ebbesson, L. O. E. (2013) Aluminum exposure impacts brain plasticity and behavior in Atlantic salmon. *Journal of Experimental Biology* 216: 3146-3155.

Chaby, L., Cavigelli, S. A., White, A., Wang, K. & Braithwaite, V. A. Long-term changes in cognitive bias and coping response as a result of chronic unpredictable stress during adolescence. *Frontiers in Human Neuroscience* 7, 328, 1-10.

Braithwaite, V. A., Huntingford, F. A. & van den Bos, R. (2013) Variation in emotion and cognition among fishes. *Journal of Agricultural and Environmental Ethics* 26: 7-23

Millot, S., Nilsson, J., Fosseidengen, J. E., Fernö, A. Braithwaite, V. A. & Kristiansen, T. S. (2013) Innovative behaviour in fish: Atlantic cod can learn to use an external tag to manipulate a self-feeder. *Animal Cognition* doi: 10.1007/s10071-013-0710-3

Ebbesson, L. O. E. & Braithwaite, V. A. (2012) Environmental impacts on fish neural plasticity and cognition. *Journal of Fish Biology* 81:2151-2174.

Archard, G., Earley, R., Hanninen, A. & Braithwaite, V. A. (2012) Correlated behavior & stress physiology in fish exposed to different predation pressure. *Functional Ecology* 26: 637-645.

Folkedal, O., Stien, L.H., Torgersen, T., Oppedal, F., Olsen, R.E., Fosseidengen, J.E., Braithwaite, V.A. & Kristiansen, T.S. (2012) Food anticipatory behaviour as an indicator of stress response and recovery in Atlantic salmon after exposure to acute temperature fluctuation. *Physiology & Behavior* 105: 350-356.

Eriksen, M. S., G. Færevik, G., Kittilsen, S., McCormick, M. I., Braastad, B. O., Damsgård, B., Braithwaite, V. A. & Bakken, M. (2011) Stressed mothers- troubled offspring? A study of behavioural maternal effects in farmed Atlantic salmon. *Journal of Fish Biology* 79: 575-586.

Moberg, O., Braithwaite, V.A., Jensen, K. H. & Salvanes, A. G. V. (2011) Assessing the effects of enrichment and food availability on the foraging behaviour of juvenile Atlantic cod. *Environmental Biology of Fishes* 91: 449–457.

Salvanes, A. G. V., Utne-Palm, A. C., Currie, B. & Braithwaite, V. A. (2011) Behavioural and physiological adaptations of the bearded goby, a key fish species of the extreme environment of the northern Benguela upwelling. *Marine Ecology Progress* Series 425: 193-202.

Archard, G. A. & Braithwaite, V. A. (2011) Increased exposure to predators increases both exploration and activity. *Journal of Fish Biology* 78: 593-601.

Archard, G. A. & Braithwaite, V. A. (2011) Population and sex differences in *Brachyrhaphis episcopi* responses to mirror images. *Behavioural Processes* 86: 52-57.

Utne-Palm, A. C., Salvanes, A. G. V., Currie, B., Kaartvedt, S., Nilsson, G. E., Braithwaite, V. et al. (2010) Trophic structure and community stability in an overfished ecosystem. *Science* 329: 333-336.

Strand, D. Utne-Palm, A.C., Jakobsen, P. J., Jensen, K. H., Braithwaite, V. A. & Salvanes, A. G. V. (2010) Enrichment promotes learning in fish. *Marine Ecology Progress Series* 412: 273-282.

NAME **CAVIGELLI**, Sonia A.	POSITION TITLE Assistant Professor Biobehavioral Health Department
eRA COMMONS USER NAME s-cavigelli	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Grinnell College	B.A.	1986-1990	Psychology/Biology
Duke University	Ph.D.	1992-1998	Psychology
University of Chicago	Post-Doc	1999-2004	Psychology

A. Positions & Honors

Positions:

1991-1992 Senior Research Assistant, Department of Psychology, Brandeis University
1992-1998 Research Assistant & Teaching Assistant, Department of Psychology, Duke University
1997 Instructor, Department of Psychology, Duke University
1997 Instructor, Department of Psychology, Guilford College
1999-2004 Research Scientist & Post-Doctoral Fellow, Dept. of Psychology, Institute for Mind & Biology, University of Chicago
2004- Assistant Professor, Dept. of Biobehavioral Health and The Huck Life Sciences Institute, and Graduate Faculty in the Neuroscience Program, Pennsylvania State University

Other Experience and Professional Memberships:

1994- Member, Society for Behavioral Neuroendocrinology
2000- Member, Psychoneuroimmunology Research Society
2005- Member, Society for Neuroscience
2005- Member, International Society for Developmental Psychobiology
2009 NIH Peer Review Committee: National Cancer Institute (NCI) Special Emphasis Panel, "Small Grants for Behavioral Research in Cancer Control"
2010 NIH Peer Review Committee: National Institute of Mental Health (NIMH) Special Emphasis Panel, "Identification and Characterization of Sensitive Period for Neurodevelopment in Studies of Mental Illnesses"

Honors:

1993 Sigma Xi, Grants-in-Aid of Research Award
1993 Duke University Dean's Summer Travel Award
1995 Center for International Studies Graduate Award
1995 Sigma Xi, Sally Hughes-Schrader Travel Award
1996 Aleane Webb Dissertation Award
1997 Animal Behaviour Society Research Award
2001 Pew Midstates Science and Mathematics Consortium
2001 NSF Travel Grant to attend 27th International Ethological Conference in Tübingen, Germany
2001-2004 Research Scientist, Institute for Mind & Biology, University of Chicago
2003 Trainee at Eleventh Annual Summer Training Course in Experimental Aging Research

B. Selected peer-reviewed publications (last 10 years))

Cavigelli, S.A (2005). Animal personality and health. *Behaviour*, 142: 1223-1244.

Cavigelli, S.A., Monfort, S.L., Whitney, T.W., Mechref, Y.S., Novotny, M., McClintock, M.K. (2005). Frequent serial rat fecal corticoid measures reflect circadian and ovarian corticosterone rhythms. *Journal of Endocrinology*, 184: 153-163.

Mateo, J.M., Cavigelli, S.A. (2005). A validation of extraction methods for non-invasive sampling of glucocorticoids in free-living ground squirrels. *Physiological & Biochemical Zoology*, 78: 1069-1084.

Cavigelli, S.A., Yee J.R., McClintock, M.K. (2006). Infant temperament predicts life span in female rats that develop spontaneous tumors. *Hormones & Behavior*, 50: 454-462.

Cavigelli, S.A., Guhad, F.A., Ceballos, R.M., Whetzel, C.A., Nevalainen, T., Lang, C.M., Klein, L.C. (2006). Fecal corticoid metabolites in aged male and female rats after husbandry-related disturbances in the colony room. *Journal of the American Association for Laboratory Animal Science*, 45: 42-46.

Cavigelli S.A., Stine M.M., Kovacsics C., Jefferson A., Diep M.N., Barrett C.E. (2007). Behavioral inhibition and glucocorticoid dynamics in a rodent model. *Physiology & Behavior*, 92: 897-905.

Cavigelli, S.A., Bennett, J.M., Michael, K.C., Klein, L.C. (2008). Female temperament, tumor development and life span: Relation to glucocorticoid and tumor necrosis factor alpha levels in rats. *Brain, Behavior and Immunity*, 22: 727-735

Boggiano, M.M., Cavigelli, S.A., Dorsey, J.R., Kelley, C.E.P., Ragan, C.M.*, Chandler-Laney, P.C. (2008). Effect of a cage divider permitting social stimuli on stress and food intake in rats. *Physiology & Behavior*, 95: 222-228.

Yee, J.R., Cavigelli, S.A., Delgado, B., McClintock, M.K. (2008). Reciprocal affiliation among adolescent rats during a mild group stressor predicts mammary tumors and lifespan. *Psychosomatic Medicine*, 70: 1050-1059.

Thanos, P.K., Cavigelli, S.A., Michaelides, M., Olvet, D.M., Patel, U., Diep, M.N., Volkow, N.D. (2009). A non-invasive method for detecting the metabolic stress response in rodents: Characterization and disruption of the circadian corticosterone rhythm. *Physiological Research*, 58: 219-228.

Cavigelli, S.A., Ragan C.M., Michael K.C., Kovacsics C.E., Bruscke A.P. (2009). Stable behavioral inhibition and glucocorticoid production as predictors of longevity. *Physiology & Behavior*, 98: 205-214.

Hermes G.L., Delgado B., Tretiakova M., Cavigelli S.A., Krausz T., Conzen S.D., McClintock M.K. (2009). Social isolation dysregulates endocrine and behavioral stress while increasing malignant burden of spontaneous mammary tumors. *Proceedings of the National Academy of Sciences*, 106: 22393-22398.

Chaby, L., Cavigelli, S. A., White, A., Wang, K. & Braithwaite, V. A. Long-term changes in cognitive bias and coping response as a result of chronic unpredictable stress during adolescence. *Frontiers in Human Neuroscience* 7, 328, 1-10.

NAME Patricia Sue Grigson	POSITION TITLE Professor
eRA COMMONS USER NAME psgrigson	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Elizabethtown College, Elizabethtown, PA	B.A.	1984	Psychology
Rutgers University, New Brunswick, NJ	M.S.	1987	Psychology
Rutgers University, New Brunswick, NJ	Ph.D.	1990	Psychology
Penn State College of Medicine, Hershey, PA	Postdoc.	1990-93	Behavioral Neurosci.

A. Positions and Honors.

1990-1993 Postdoctoral Fellow Penn State University, College of Medicine, Hershey, PA
1993-1995 Sr. Research Associate Penn State University, College of Medicine, Hershey, PA
1995-2000 Assistant Professor Penn State University, College of Medicine, Hershey, PA
2000-2007 Associate Professor Penn State University, College of Medicine, Hershey, PA
2007-present Professor Penn State University, College of Medicine, Hershey, PA

Honors and Awards.

1990-1993 Postdoctoral National Research Service Award
May, 2000 Recipient of the Annual Hinkle Society Junior Investigator Award
July, 2004 Recipient of the Alan N. Epstein Research Award, Society for the Study of Ingestive Behavior

B. Selected peer-reviewed publications (last 10 years).

Liu, C. & Grigson, P.S. (2005). Brief access to sweets protect against relapse to cocaine-seeking. *Brain Research*, 1049(1):128-31.

Twining, R.C., Hajnal, Han, L., Bruno, K., Hess, E.J., & Grigson, P.S. (2005). Lesions of the ventral tegmental area disrupt drug-induced appetite stimulating effects but spare reward comparison. International *Journal of Comparative Psychology*, 18: 372-396.

Liu, C., & Grigson, P.S. (2005). u-Opioid receptor agonist DAMGO-induced suppression of saccharin intake in Lewis and Fischer rats. Brain Research, 1064: 155-160.

Freet, C.S., Tesche, J.D., Tompers, D.A., Riegel, K.E., & Grigson, P.S. (2006). Lewis rats are more sensitive than Fischer rats to successive negative contrast, but less sensitive to the anxiolytic and appetite stimulating effects of chlordiazepoxide. *Pharmacology Biochemistry & Behavior*, 85: 378-384.

Grigson, P.S., & Hajnal, A. (2007). Once is too much: Conditioned changes in dopamine following a single saccharin-morphine pairing. *Behavioral Neuroscience*, 121: 1234

Kuntz, K.L., Twining, R.C., Baldwin, A.E., Vrana, K.E., & Grigson, P.S. (2008). Heroin self-administration: I. Incubation of goal-directed behavior in rats. Pharmacology Biochemistry & Behavior, 90(3): 344-348

Grigson, P.S., Twining, R.C., Freet, C.S., Wheeler, R.A., & Geddes, R.I. (2008). Drug-induced suppression of CS intake: Reward, aversion, and addiction. In: *Conditioned Taste Aversion: Behavioral and Neural Processes, 74-91. S. Reilly & T. Schachtman, (Eds). Oxford University Press. NY, NY.*

Geddes, R.I., Han, L., Baldwin, A.E., Norgren, R., & Grigson, P.S. (2008). Gustatory insular cortex lesions disrupt drug-, but not LiCl-, induced suppression of CS intake. Behavioral Neuroscience, 122: 1038-1050.

Grigson, P.S. (2008). The State of the reward comparison hypothesis: Theoretical comment on Huang and Hsiao. Behavioral Neuroscience, 122: 1383-1390.

Liu, C., Showalter, J., & Grigson, P.S. (2009). *Ethanol-Induced Conditioned Taste Avoidance: Reward or Aversion? Alcohol Clin Exp Res Dec 19 [Epub ahead of print]*.

Freet, C.S., Steffen, C., Nestler, E.J., & Grigson, P.S. (2009). Over expression of ΔFosB is associated with attenuated cocaine-induced suppression of saccharin intake in mice. *Behavioral Neuroscience*, 123(2): 397-407.

Twining, RC., Bolan, M., & Grigson, P.S. (2009). Yoked delivery of cocaine is aversive and protects against the motivation for drug in rats. *Behavioral Neuroscience*, 123: 913-925.

Puhl, MD., Fang, J., Grigson, P.S. (2009). Acute sleep deprivation increase the rate and efficiency of cocaine self-administration, but not the perceived value of cocaine reward in rats. Pharmacology Biochemistry and Behavior, 94:262-270.

Corwin, R.L., Grigson, P.S. (2009). Symposium overview-food addiction: Fact or fiction? *J of Nutrition*, 139: 617-619.

Grigson, PS. Reward Comparison: The Achilles' heel and hope for addiction. *Drug Discov Today Dis Models.* 2009; 5(4):227-233.

Puhl MD, Cason AM, Wojnicki FH, Corwin RL, Grigson PS. A history of bingeing on fat enhances cocaine seeking and taking. Behav Neurosci. 2011 Dec; 125(6):930-42.

Liang NC, Grigson PS, Norgren R. Pontine and thalamic influences on fluid rewards: II. Sucrose and corn oil conditioned aversions. Physiol Behav. 2012 Jan 18; 105(2):589-94.

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Puhl MD, Blum JS, Acosta-Torres S, Grigson PS. Environmental enrichment protects against the acquisition of cocaine self-administration in adult male rats, but does not eliminate avoidance of a drug-associated saccharin cue. Behav Pharmacol. 2012 Feb; 23(1):43-53.