E. Other Support (to PI and Co-I)

Flexibility, prefrontal function, and normal aging
R01 AG027097, 9/1/07-08/31/12
Role: PI ADC: ~150,000

The goal of this project is to identify neural correlates of aging-related declines in reversal-learning and set-shifting in orbitofrontal and medial prefrontal cortex.

Role of orbitofrontal cortex and outcome expectancies in associative learning
R01 MH080865, 6/1/08-5/31/13
Role: PI ADC: ~200,000

The goal of this project is to identify a specific role for outcome expectancies signaled by the orbitofrontal cortex in changing behavior in the face of unexpected outcomes.

Corticolimbic encoding of conditioned reinforcers: relevance to addiction
R01 DA015718, 12/1/08-11/30/2013
Role: PI ADC: ~250,000

The goal of this project is to identify the associative and circuit basis of conditioned reinforcers.

Errors and expectancies in OFC and VTA and their roles in associative learning
R01 MH084711, 6/1/09-5/31/11
Role: PI ADC: ~250,000

The goal of this project is to use blocking combined with single-unit recording and inactivation to test the hypothesis that OFC interactions with error signals in VTA to drive learning.

NONE OF THESE PROJECTS HAVE ANY OVERLAP WITH THE PROPOSED EXPERIMENTS. INDEED THE PROPOSED WORK IS OUTSIDE THE SCOPE OF ALL OF THE GRANTS ABOVE.

F. Resources and Environment

The lab has neurophysiology and behavioral facilities (~2000 sq ft) for this research located in the Department of Anatomy and Neurobiology in the HSF-1 Building. Ample office space is located in the HSF-1 building on the same floor as the labs. AAALAC accredited animal facilities sufficient for the experiments in this proposal are available in the Howard Hall building connected to HSF-1.

Equipment in lab includes two stereotaxic surgical setups with gas anesthesia and articulating scopes. A sliding microtome, cryostat, fume hood, microscopes and cameras are also available. In addition, there are six rooms equipped with 11 custom-built behavioral training systems/boxes appropriate for the current experiments, 9 of which are equipped with complete Plexon recording setups and 2 equipped with equipment for measuring dopamine release using fast-scan cyclic voltametry. Offline Sorter and Neuroexplorer from Plexon, Statistica, and Matlab are available on networked workstations described above for sorting and analysis of neural data. In addition, 8 custom-built training boxes that are identical in all respects to those in the recording setups are available for training rats before recording and for non-recording experiments, and an additional 40 commercial training boxes from Coulbourn Instruments, similar in most respects to those used for recording are also available for behavioral experiments that do not involve recording.

The laboratory is equipped with high-end workstations for analyzing neural data, as well as machines for acquiring data, creating graphics and preparing data for publication. All computers are networked and firewalled to allow ease of collaboration and sharing within the lab. A central server maintains a data/backup archive that is available from on and off-campus 24 hours per day and a VPN that allows remote access to our network accessible software licenses.

G. Background

Schizophrenia is a chronic brain disorder that affects about 1.1 percent of adults in the United States. Impaired reality testing, in which internal beliefs can distort or become confused for reality, is a prominent and devastating symptom of schizophrenia. Understanding the neural dysfunction underlying impaired reality testing would be a significant step towards restoring normal function to schizophrenic patients but is hindered by the fact that, to the best of our knowledge, no behavioral means of assessing impaired reality testing in animals exists. The proposed experiments will use the NVHL rat combined with a task termed representation-mediated taste aversion (RMTA) to develop a model of impaired reality testing in rats. The development of such a model – along with the enhanced understanding of how the normal brain distinguishes real from imagined representations that will come from studying the underlying basis of RMTA - will promote our understanding of impaired reality testing in schizophrenia and other diseases. Additionally by validating the NVHL model, the proposed studies will provide a potential vehicle in which pre-clinical testing of drugs and other therapies may be more rapidly conducted, as well as supporting the general utility of this important animal model for studying other aspects of schizophrenia Finally by providing a valid animal model of such an abstract human capacity, the proposed work will demonstrate the value of animal models – particularly rodent models – for studying even the most complex and apparently unique human capabilities.

Our approach hinges on two tools. The first is the neonatal ventral hippocampal lesioned (NVHL) rat. The NVHL rat is a highly studied, neurodevelopmental model of schizophrenia. Its widespread use is due in part to the similarity between the behavioral syndrome of NVHL rats and the symptoms of schizophrenia, as well as similarities in physiological end points [31]. Like schizophrenic patients, NVHL rats show onset of positive-like symptoms and changes in prefrontal dopamine function towards late puberty/young adulthood. However, there has been no attempt to demonstrate impaired reality testing – a hallmark of schizophrenia – in NVHL rats. Such a demonstration will dramatically improve the face validity of this model for clinical researchers who might benefit from its findings.

The second tool – which we will use to assess reality testing in the NVHL's – is a Pavlovian training procedure termed representation-mediated taste aversion or RMTA. This procedure takes advantage of the empirical observation that, when a hungry rat is trained that a cue predicts a food reward, the cue comes to evoke an internal representation of reward [12-14]. This representation likely occurs through activation of some subset of the neural network activated by the reward itself [2, 9, 22, 26]. Interestingly, in normal adult rats, this cue-evoked representation of the food can, briefly, substitute for actual food. This can be shown by pairing the cue with illness (nausea). Under the proper conditions, this will produce an aversion to the food [3, 4, 11, 15], just as if the food was directly paired with illness [7]. This phenomenon has been termed a representation-mediated taste aversion (RMTA), since it must be "mediated" through the cue-evoked or imagined neural representation of the food.

Put simply, RMTA represents in essence a failure to fully distinguish an internal representation from reality – a form of impaired reality testing. Obviously this is not normal or even particularly adaptive behavior. Accordingly, in normal adult rats, RMTA is highly transient, disappearing after minimal cue-food training [16]. This suggests that a neural mechanism exists for rapidly distinguishing or dissociating the neural ensemble that is triggered by actual food presentation and that which becomes associated with (and triggered by) the predictive cue.

For example, imagine that a food reward, such as sucrose, evokes a strong response in an ensemble of 100 neurons. This would be the neural representation of sucrose delivery, in a particular context. If sucrose is reliably predicted by a cue in that context, the same 100 neurons might initially become active during the predictive cue, perhaps even recapitulating the sucrose-evoked pattern of activity (the ensemble state). Activation of the sucrose ensemble during the cue would be, in essence, a neural instantiation of the Pavlovian cue-outcome association, capable of supporting outcome-guided behaviors. In fact, we have shown the development of such cue-outcome encoding ensembles in the orbitofrontal cortex [27, 28], a prefrontal area critical to outcome-guided behavior [1, 6, 17, 19, 21, 24].

It stands to reason if the neural ensemble triggered by the cue shares too many features – ie neurons or perhaps pattern of neural activity– with the neural ensemble initially responsive to actual sucrose delivery, then the animal might mistake the imagined sucrose for the real thing. Thus it is important, at some level, to dissociate or separate the neural ensemble state that is evoked by the predictive cue from that evoked by the actual reward. This could happen by simply tuning the cue-evoked ensemble, to reduce the similarity in the two populations, essentially eliminating irrelevant aspects of the representation that are consistent only with the actual presence of the reward. This would appear as a decoupling or reduced correlation between the ensemble state at the time of reward and that evoked by the cue with training. Such a mechanism for tuning or sculpting prefrontal cortex activity has recently been proposed; in this proposal the critical sculpting is proposed to depend on dopamine-dependent inhibition of prefrontal activity [8]. Notably dopamine-dependent inhibitory function in prefrontal areas is diminished in NVHL rats [10, 30].

The proposed experiments will build on the ideas described above regarding the theoretical and neural basis of RMTA to develop a model of impaired reality testing with relevance to schizophrenia. In Aim 1, we will test the hypothesis that RMTA is abnormally prolonged in NVHL rats; this basic result is already supported by preliminary data from our lab (see below). We will build on this work to show that the prolonged RMTA in NVHL rats is reversed by antipsychotic agents known to improve reality testing in schizophrenic patients. For this, we will use clozapine, an atypical antipsychotic that has been shown to effectively treat pre-pulse inhibition in both schizophrenic patients [23] and NVHLs [20, 25]. In Aim 2, we will use single unit recording combined with a sophisticated approach to identifying neural representations in real time activity of small neural ensembles to show that RMTA reflects the initial overlap between the cue-evoked imagined representation of the food outcome and the actual representation. Further we will test the hypothesis that the disappearance of RMTA in controls (and its persistence in NVHL rats) reflects the separation (or failed separation) of the imagined and actual representations in orbitofrontal cortex.

H. Specific Aims

Specific Aim 1 – Determine the efficacy of clozapine on RMTA in sham and NVHL rats

Hypothesis: *Neonatal ventral hippocampal lesions will enhance and prolong RMTA; treatment with clozapine, an atypical antipsychotic, will restore behavior to normal*

To test this hypothesis, sham and NVHL rats will be trained in RMTA after treatment with clozapine or saline. Rats will receive varying amounts of training on the initial cue-reward association.

Expected Outcomes:

- NVHL rats will show more robust and/or prolonged RMTA than controls (see preliminary data).
- Treatment with clozapine prior to and during training will normalize RMTA in NVHL rats.

Specific Aim 2 – Determine the relationship of cue-outcome activity in orbitofrontal cortex to the expression of RMTA in sham and NVHL rats

Hypothesis: *RMTA reflects a transient failure to dissociate the neural populations coding imagined versus real reward; this dopamine-dependent process of dissociating the developing representation of the imagined or expected reward from real reward is impaired in NVHL rats, leading to enhanced and prolonged RMTA*

To test this hypothesis, we will record neural activity from small populations of neurons in the orbitofrontal cortex, an area critical to signaling information about expected outcomes, in sham control and NVHL rats. Recordings will be made during training in the RMTA task. Using both conventional approaches to the analysis of single-units [10, 29] and ensemble analyses based on a Hidden Markov Model [5, 18], we will examine the correspondence between the reward-evoked neural population and the cue-evoked response in rats at various points in training.

Expected Outcomes:

- NVHL rats will show more robust and/or prolonged RMTA than controls (see preliminary data).
- In controls, activity in individual neurons or across small ensembles during the reward and the cue will be poorly correlated initially in training, this correlation will increase rapidly with training on the cue-reward association, and then decline to an intermediate value. Over all subjects, the peak of correlation will correspond to the time of highest RMTA; in individual rats, RMTA will be tested at different points in training, however we still expect to see correspondence between the neural correlation at the time of testing and the RMTA.
- In NVHL rats, we expect to see higher and more prolonged correlated activity, particularly in those neurons or neural ensembles that are active to the reward initially. Correlations will again correspond to the pattern of RMTA displayed by individual rats.
- Time permitting, we will also test whether clozapine treatment restores the normal pattern of correlation between neural activity at the time of the cue and at the time of reward in NVHL rats

I. Preliminary Results

Figure 1 presents preliminary data from RMTA in sham (n=16) and NVHL (n=16) rats. All rats were trained to associate a light with delivery of a sucrose pellet. In the mediated taste aversion phase unpaired rats (n=8 rats per lesion condition) received light and nausea in separate sessions while paired rats (n=8 rats per lesion condition) received light and nausea in the same session. A comparison of sucrose consumption before and after the light-nausea pairing, shown in the lower part of the figure, demonstrated that only NVHL-Paired rats formed an aversion to sucrose. Sham and NVHL rats conditioned similarly and showed identical performance in a conventional reinforcer devaluation task (in which the food is directly paired with illness; data not shown). Thus the effect is not attributable to abnormal learning or perception of reward or illness; instead NVHL rats were specifically impaired in their ability to distinguish the imagined representation of sucrose from actual sucrose. Further work in our lab, not shown in such limited space, revealed that the effect in NVHL rats is present after only 6 light->sugar pairings and persists for as long as 24 pairings, a duration which far exceeds that observed in normal rats by us or other investigators [3, 4, 11, 15].

J. Methods of Procedure

The proposed experiments will use the RMTA task outlined above with methods already in use in the Schoenbaum lab or in the labs of our consultants, Drs O'Donnell and Katz. This includes the RMTA task, as well as the techniques to create NVHL rats [10, 30], record small ensembles of neurons in the prefrontal cortical regions [10, 29], and to analyze ensembles of simultaneously recorded neurons using a Hidden Markov Model to identify neural states encoding imagined and real rewards [5, 18].

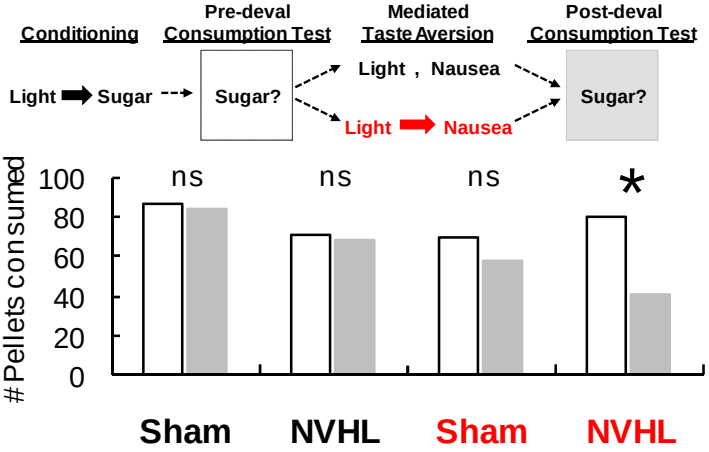

Figure 1. Experimental outline (Top) and critical consumption data for RMTA (Bottom). Sham- and NVHL-unpaired rats received light and illness in separate sessions while paired rats (in red) received light and illness in the same session. Pairwise comparisons found Sham-Unpaired, NVHL-Unpaired and Sham-Paired rats failed to form a taste aversion (ns, ps>0.1). NVHL-Paired rats demonstrated a significant taste aversion (*p=0.016).

K. Scientific Significance and Relevance for NARSAD Mission

A major goal of NARSAD is to support the development of approaches to the treatment of neuropsychiatric disorders, particularly schizophrenia. Animal models are clearly critical to this effort, as they allow researchers to test hypotheses about the underlying causes of various symptoms and how to address them therapeutically without incurring major costs or putting at risk human patients. While some aspects of schizophrenia have been successfully modeled, either by the NVHL rats or other models, the cardinal feature of schizophrenia – impaired reality testing – has not been recreated. Our proposal offers the potential to address this shortcoming. This will have at least three clear benefits. First and foremost, it will provide an assay in which one could test new therapies for schizophrenia. Although current drugs do a reasonable job of correcting the positive symptoms in schizophrenia, they often have significant side effects. Thus better drugs are called for. Further agents that are developed to address negative symptoms must also be evaluated for their effect on the positive symptoms. Our work would provide a valid animal model for such testing. Second our work will also strengthen the NVHL model of schizophrenia. This model has been successful in explaining many aspects of schizophrenia. Validation would aid in this model's adoption by the scientific community. Finally we believe that the demonstration that one can model impaired reality testing in animals – rodents – will provide strong support for the general use of animal models in understanding neuropsychiatric disease. Broader acceptance and use of such models will be beneficial for advancing our understanding of these diseases.

L. References

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M. Human Subjects - NA