Limbic Activation to Novel versus Familiar Food cues Predicts Food Preference and Alcohol Intake[*]

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Abstract

Expectation of salient rewards and novelty seeking are processes implicated in substance use disorders but the neurobiological substrates underlying these associations are not well understood. To better understand the regional circuitry of novelty and reward preference, rats were conditioned to pair unique cues with bacon, an initially novel food, or chow, a familiar food. In the same animals, after training, cue-induced brain activity was measured, and the relationships between activity and preference for three rewards, the conditioned foods and ethanol (EtOH), were separately determined. Activity in response to the food paired-cues was measured using brain glucose metabolism (BGluM). Rats favoring bacon-paired (BAP) cues had increased BGluM in mesocorticolimbic brain regions after exposure to these cues, while rats favoring chow-paired (CHP) cues showed relative deactivation in these regions. Rats exhibiting BAP cue-induced activation in prefrontal cortex (PFC) also consumed more EtOH while rats with cortical activation in response to CHP cues showed lower EtOH consumption. Additionally, long-term stable expression levels of PFC *Grin2a*, a subunit of the NMDA receptor, correlated with individual differences in EtOH preference insomuch that rats with high EtOH preference had enduringly low PFC *Grin2a* mRNA expression. No other glutamatergic, dopaminergic or endocannabinoid genes studied showed this relationship. Overall, these results suggest that natural variation in mesocorticolimbic sensitivity to reward-paired cues underlies behavioral preferences for and vulnerability to alcohol abuse, and support the notion of common neuronal circuits involved in food- and drug-seeking behavior. The findings also provide evidence that PFC NMDA-mediated glutamate signaling may modulate these associations.

Abstract Word Count: 250

Key Words: behavioral imaging, positron emission tomography, neuroimaging, craving, reward, rat

1. Introduction

Personality traits associated with novelty seeking have been consistently associated with greater vulnerability for substance use disorders including alcoholism (Finn, 2002). Indeed differences in expectation modify the sensitivity to reward and preferences (i.e. food or drugs) (Hurling and Shepherd, 2003; Lee et al., 2006; Plassmann et al., 2008; Tuorila et al., 1994). Furthermore, an individual's expectation of future outcomes mediating approach/avoidance behavior is central to the treatment of substance use disorders. Therefore, understanding the effects of expectation on brain activity, and whether these effects predict related behaviors in the future, is critically important.

Human imaging studies have assessed the effects of expectation on behavior in the healthy (Petrides, 2007) and diseased brain (Volkow et al., 2010). These studies showed that in the addicted brain, prior drug history modifies the impact of drug-expectation on regional brain activity. Specifically while in non-drug abusers, expectation of receiving a stimulant drug – with effects similar to cocaine – activated regions involved with emotional reactivity and reward, in cocaine abusers, stimulant treatment activated regions involved in arousal (Volkow et al., 2003; Volkow et al., 2006). Clinical studies, however, are limited by their inability to control for inter-subject variability due to distinct genetic and environmental factors (i.e. including past experiences with the reward), and thus the use of preclinical models allows investigators to evaluate expectation with control over these and other factors.

Here, using a rodent behavioral neuroimaging approach, we assessed the regional brain activity associated with the "expectation" of two distinct foods that, like the aforementioned human studies, differed in terms of familiarity (novel versus familiar). To test if behaviors related to food- and drug-seeking engage common neural circuitry, we also assessed whether brain activity to the novel food-cue predicts future ethanol (EtOH) preference, a novel appetitive stimulus with established abuse-potential. To accomplish these tasks, animals were first trained to associate foods with distinct cues using conditioned place preference (CPP), and after training, preference for each chamber was assessed (Figure 1A, see Methods). On separate days thereafter, brain activity in response to each chamber – with chamber-cues only – was measured using FDG and and small animal positron emission tomography (μPET) to assess regional brain-glucose metabolism (BGluM) (Figure 1B, see Methods). We hypothesized that cue-induced activity in regions involved in reward learning would predict CPP for the paired food. As bacon and EtOH share several attributes in this study, namely they are both novel rewards, we hypothesized that brain activity, in response to the bacon-paired cues, would also predict future EtOH consumption.

2. Results

Technical shortcomings led to failed μPET acquisition in three animals and failed mRNA measures in one animal so that while twelve animals were included in the behavioral analyses, only nine are reported in the regressions with BGluM and eight in the regressions with mRNA data.

2.1 Food-predictive cues induce reproducible conditioned preference responses in rats

Regarding daily food consumption during CPP-conditioning, rats consumed significantly more bacon than chow on the first (P=0.03) and third (P=0.003) conditioning sessions (Figure 2A). On average all rats consumed more bacon (2.44 ± 0.22 g) than chow (1.4 ± 0.32 g) (P=0.008). We did not find a significant CPP for bacon-paired (BAP) cues at the group-level, due to variability between the animals in their preferences for BAP or for chow-paired (CHP) chambers. Although there was no difference in mean preference, there was a statistically significant difference in the spread (overall variance) of responses between the pretest and test sessions (F-test) (TD1: P=0.005, TD2: P=0.002) but not between the two test sessions (P=0.83) (Figure 2B). Before conditioning, each animal showed little preference for a particular chamber, evident by the tight clustering of values around the indifference point (dashed line, Figure 2B), however after conditioning, there was greater variation in these values, suggesting that most animals spent more time in a particular chamber. These conditioned preferences were reproducible, evident by the significant correlation between Test Days 1 and 2 (r=0.68, P=0.04), and the segregation of gray-scaled points shown in Figure 2B. However, there were no differences in bacon consumption during conditioning trials between the rats that showed CPP for BAP cues and those that showed CPP for CHP cues, indicating a disassociation between the incentive aspects of bacon consumption and the conditioning responses linked with expectation.

2.2 Individual differences in brain activation during exposure to food-paired cues predict preference for these cues

We found no significant group-level differences in BGluM when rats were placed in the BAP chamber, relative to the CHP chamber. For both bacon and chow, brain activity in response to food-paired cues positively correlated with preference for that food (Figure 3). The correlations, shown for bacon relative to chow, were significant in the NAc (P=0.004), VP (P=0.007), HYP (P=0.002), CPu (P=0.01), OR (P=0.04), TH (P=0.03), VMB (P=0.04) and VPo (P=0.03). Similar correlations were obtained when using the preferences obtained for Day 2 (except in VPo and VMB) (data not shown), but importantly, these correlations were not observed with pretest preference, suggesting that changes in brain activity were not due to intrinsic preferences for the chambers *per se*.

2.3 Individual differences in brain activation during exposure to food-paired cues predict EtOH consumption

Cue-induced BGluM in the PFC, in response to BAP cues, positively correlated with 8% EtOH intake (P=0.02) and preference (P=0.01, Figure 4A). No significant regressions between EtOH intake, or preference, and PFC BGluM were observed at the other EtOH concentrations.

2.4 Individual differences in EtOH consumption predict variation in Grin2A gene expression in prefrontal cortex

We examined correlations between the relative PFC expression of the nine *a priori* selected genes and both EtOH intake and preference. For cortical *Grin2A*, significant negative correlations were observed with 8% EtOH intake (r=0.81, P=0.02) and preference (r=0.84, P=0.02, Figure 4B). In addition, *Grin2A* mRNA expression was negatively correlated with 4% (r=0.8, P=0.03) and 6% (r=0.8, P=0.03) EtOH intake (not shown). There was no significant relationship to the other glutamatergic, dopaminergic and endocannabinoid genes studied.

3. Discussion

We measured regional brain activity after presentation of contextual cues that predicted two distinguishable foods – bacon, which was novel and chow, which was familiar. This was accomplished by pairing the delayed receipt of bacon, or chow, with specific cues (BAP and CHP cues, respectively), and then using *in vivo* behavioral imaging to measure brain activity in response to these cues (cue-induced BGluM). Although there were no differences in bacon intake between the two groups, some rats spent more time in the BAP chamber whereas others spent more time in the CHP chamber. This variation was consistently reproduced on two separate test sessions, suggesting that it represented true individual differences in preference for BAP or CHP cues. Interestingly, food intake throughout conditioning did not predict cue-preference or cue-induced changes in BGluM, suggesting a disassociation between the rewards' incentive for its consumption, measured by intake, and their conditioned associations, measured by CPP. Consistent with the complex nature of reward, which included conditioning and incentive motivation components, cue-induced BGluM and cue preference correlated, while these did not predict an animal's specific consumption of the food *per se*.

Our behavioral imaging paradigm identified a network of brain regions in the rat previously implicated with human reward-expectation (Coletta et al., 2009; DelParigi et al., 2005; Volkow et al., 2010). We found that regional limbic BGluM activity during food-cue exposure significantly correlated with food-cue preference. That is, certain rats showed increases in BGluM in the OR, VP, NAc, CPu, VMB, VPo, TH, and HYP with exposure to BAP cues, while other rats showed decreases in BGluM in these regions upon BAP cues exposure. Intriguingly, the rats that showed increases in BGluM during BAP cue exposure also showed greater preference for the BAP chamber, while rats that showed decreased BGluM during BAP cue exposure showed low preference for this chamber. The two foods used for conditioning differed not only with respect to the history of exposure (lifetime exposure in the case of chow), but also in their unique somatosensory characteristics, which would have further enhanced the novelty of the experience including flavor, temperature (bacon was served warm whereas chow was room temperature) and caloric content. A combination of these factors, primarily novelty, likely increased the saliency of bacon relative to chow, which had a more positive impact on a subset of rodents, namely the bacon-preferring animals, due to inherent variation in personality traits.

The brain areas that were identified in our behavioral imaging task have also been implicated in brain circuits underlying drug anticipation, reward and craving (Koob and Volkow, 2010). Thereby, the brain activation exemplified in this report, and elsewhere (Volkow *et al.*, 2011), suggest that overlapping brain circuitry encodes for conditioning to food and conditioning to drugs. Based on this, we hypothesized that rats showing stronger brain responses to a novel, more salient food (bacon being a more salient stimulus over chow) would show a greater propensity to drink EtOH than those that showed greater activation for a familiar and less salient food-cue. Indeed, EtOH intake and preference significantly correlated with BGluM in PFC during exposure to food-predictive cues. In particular, rats that consumed greater quantities of EtOH and that showed greater preference for EtOH over water, showed greater BGluM in PFC with exposure to BAP cues, while rats with lower EtOH intake and preference showed decreased BGluM in PFC with exposure to BAP cues.

Our findings are reminiscent of findings from studies that classify rats into those that behaviorally respond to rewards *per se* (goal trackers) versus those that preferentially respond to cues (sign trackers) (Flagel *et al.*,

2011). According to these studies, sign trackers may be "hardwired" to respond to cues that signal incentive salience, irrespective of the nature of the reward (food, drugs, sex, etc.), while goal trackers may be "hardwired" to respond to the reward itself. Goal trackers may be less vulnerable to overconsumption of rewards since they would only experience the rewarding effects of a stimulus once attained. We predict that the rats that showed stronger brain responses to BAP cues (novel cue) are more sensitive to conditioning stimuli and therefore much more likely to be "sign trackers". On the other hand, animals that showed weaker brain responses to the BAP cue (and thus stronger brain responses to the CHP cue, which is a cue that has signaled food throughout their life), are more likely to be "goal trackers". In fact there is some evidence that "sign trackers" consume greater quantities of EtOH than goal trackers (Anderson and Spear, 2011) and rats that are more sensitive to salient food-cues (sucrose) also assign stronger incentive salience to cocaine cues (Meyer et al., 2012). However, food consumption for bacon or chow did not correlate with CPP or BGluM, which indicates that conditioning for the food cues does not predict their actual consumption. Since we did not condition rats to bacon or chow directly, but to cues that predicted bacon and chow delivery, the observed CPP to the food cues and accompanying BGluM during cue exposure is more likely to reflect the anticipation to the cues rather than their actual consummatory processes.

A month following the alcohol studies, brains were harvested and mRNA expression in the PFC was assessed, and although expression varied across the animals, levels of *Grin2A* expression (among nine different transcripts assessed) strongly predicted an individual's past EtOH intake. As these *in vitro* measurements were performed four weeks after EtOH exposure, these results are not likely to represent the direct acute actions of EtOH, or EtOH withdrawal, and instead suggest that long-term stable differences in PFC *Grin2A* expression may contribute to differences in EtOH intake. Indeed, recent studies suggest that *Grin2A* plays a role in alcohol dependence (Karpyak et al., 2011; Schumann et al., 2008). Furthermore, a polymorphism in the promoter region of *Grin2A* has been identified (Schumann et al., 2008), and the longer forms of these alleles occur more frequently in alcohol-dependent subjects, while the shorter forms occur more frequently in control subjects (Domart et al., 2011). As the longer forms are associated with reduced mRNA expression, this case-control study agrees with the negative correlation between mRNA expression and EtOH intake that our study identified, as both suggest that lower *Grin2A* levels increase predisposition to EtOH dependence.

Grin2A encodes the glutamate receptor subunit 2A (NR2A), a component of the NMDA glutamate receptor. This protein is likely involved in the induction of long-term potentiation (LTP), a correlate of learning and memory believed to play a role in the development of addiction. Our results do not allow us to make generalizations regarding NR2A's function since low *Grin2A* levels may either indicate low NR2A function due to transcriptional deficits, or a compensatory decrease secondary to high NR2A function (feedback inhibition). It is therefore not clear whether low *Grin2A* levels would be indicative of low LTP or a high LTP. We are currently exploring the relationship between NR2A function in PFC and ethanol intake. Although preliminary, our results suggest that rats showing increased ethanol intake may be characterized by efficient NR2A PFC function, which would underlie addiction susceptibility via enhanced plasticity in learning and memory mechanisms.

Limitations for this study include the limited spatial resolution of FDG μPET, which decreases our ability to accurately quantify changes in glucose metabolism in any one given region. Multiplicity of correlations was another limitation, as this increases the likelihood of false positives, yet an uncorrected threshold of $p < 0.05$ was used since we expected *a priori* relationships between activity in limbic regions and conditioned behaviors. Additionally, our experimental design included built-in controls that allowed us to distinguish between false positives and true positives. Our longitudinal approach involved multiple measurements of the same animal across time, allowing us to assess chamber-preference before and after conditioning. When we assessed correlations of ROI activity vs. chamber preference after conditioning (Test Day 1 for instance), 8 out of 24 (~33%) comparisons were significant. Although true, we did not observe any single correlation between brain-activity and preference before conditioning. These *pre-conditioning* contrasts functioned as internal controls, and the absence of any correlation strongly suggests that random chance cannot explain the correlations observed with the *post-conditioning* data. This is further supported by the finding that when we tested *post-conditioning* chamber preference again (Test Day 2), we were able to reproduce 6 out of 8 (75%) significant comparisons from Test Day 1.

To summarize, brain activity in mesocorticolimbic regions during exposure to novel versus familiar food cues predicted conditioned preference for these cues, as well as for EtOH consumption, though it was not correlated with the actual food consumption. It is generally accepted that appetitive stimuli are intrinsically rewarding, since they are ultimately necessary for survival. Reward, however, is a complex notion and is characterized by multidimensional behavioral profiles (e.g. stimulus-seeking vs. stimulus-consumption). In our case, our CPP procedure was tied to the expectation of receiving an appetitive stimulus and therefore it primarily captured the seeking dimension of reward. Since consumption captured strictly the hedonic aspects of the reward, dissociation between consumption and place-preference is an expected and explainable finding. Overall, these results suggest a functional link between the strength of the brain's response to stimulus expectation and the preference for the conditioned cues (and not the stimulus) that triggers the expectation. These findings also suggest that common brain circuits are involved in expectation for novel, salient foods, such as bacon, and alcohol, and that natural variation in preference for specific food cues predicts EtOH preference, which might be partly mediated by *Grin2A* expression in PFC.

4. Experimental Procedure

4.1 Animals

Twelve, adult male Sprague-Dawley rats (Taconic Farms, Inc., New York) were used. All rats had unlimited access to standard rat chow and tap water throughout the study. Rats were on a reverse 12-hour light cycle (2000 hrs on, 0800 hrs off). All procedures were conducted in accordance with the National Institutes of Health and Brookhaven National Laboratory Institutional Animal Care and Use Committee's guidelines (NAS and NRC, 1996).

4.2 Conditioned-Place Preference (CPP)

4.2.1 Apparatus

Rats were conditioned in a three chamber custom-made CPP apparatus. A central corridor (12 cm × 20 cm × 20 cm) linked two, equally sized conditioning chambers (30.5 cm × 20 cm × 20 cm each). The conditioning chambers were distinguished by unique wall color (white/black), wall pattern, and floor grating. All trials were conducted between 1400 and 1800 hrs under dark conditions and chambers were under video surveillance during the experiments.

4.2.2 Stimulus Acclimation and Pretest

Prior to the start of conditioning, Oscar Mayer© cooked bacon (5g) (Kraft Foods, Northfield, IL) and chow pellets (regular food) were placed in each rat's home cage. This was done twice, starting two days before conditioning, to acclimate the animals to the novelty of the bacon. On pretest day, rats were initially placed in the middle corridor, and then given unrestricted access to both conditioning chambers for 15 minutes. The amount of time spent in each chamber was calculated as the percent time spent in each of the two, conditioning chambers. If a rat preferred one chamber relative to the other (≥ 55%), bacon was paired with the non-preferred chamber while chow was paired with the preferred one and vice-versa. If the apparatus was unbiased (~50% preference), the food-assignment was randomly determined.

4.2.3 Conditioning

For eight consecutive days, each rat was separately placed in the bacon-paired (BAP) and chow-paired (CHP) chambers (4 days in each, alternating every other day); each daily session lasted 30 minutes. Rats were placed in each chamber with the lights off and 20 minutes later 5g of either chow or bacon were placed in the respective chamber so that they would consume it. Rats had free access to the food for ten minutes and the amount consumed was measured. Beginning on day five, rats received intraperitoneal (IP) injections of saline prior to being placed in the conditioning chambers, in order to acclimate them to the imaging procedure (described in the next section). The study design is illustrated in Figure 1A.

4.2.4 Testing

Place preference was assessed on two occasions, once the day after the last conditioning session and before imaging sessions (Test Day 1), and then again after the end of the imaging sessions (Test Day 2). The purpose of Test Day 2 was to assess reproducibility of CPP responses. As with pre-conditioning, on Test Days 1 and 2 rats had access to the two conditioning chambers for 15 minutes. To quantify CPP to bacon or chow, the duration of time spent in the BAP or CHP chamber on Test Days 1 and 2 was compared to the duration of time spent in both chambers and expressed as percent preference with respect to the BAP chamber. Finally, each rat was exposed to two reconditioning sessions (one for BAP and one for CHP cues) between Test Days 1 and 2 to strengthen conditioning associations that may have weakened due to the imaging sessions (during imaging sessions rats were confined to either the BAP and CHP chambers but did not receive bacon or chow).

4.3 Small Animal Positron Emission Tomography (μPET)

4.3.1 Apparatus and Procedure

The scanning procedure is illustrated in Figure 1B. We used a µPET R4 tomograph (Concorde CTI Siemens, Knoxville, TN), which has a transaxial resolution of 2.0mm full-width at half maximum with a field-of-view of 11.5 cm. Rats were scanned twice using *in vivo* µPET and 2-fluoro-2-deoxy-D-glucose (FDG) as described (Thanos et al., 2008b). In brief, scans were separated by two days and on each day, animals were injected IP with ~1 mCi FDG 30 minutes prior to image acquisition (uptake period). This *in vivo* IP dosing of FDG produces similar brain glucose uptake as intravenous (IV) administration (Schiffer *et al.*, 2007) (Meibach *et al.*, 1980). During the FDG uptake period, animals remained awake and were placed in either the BAP or CHP chamber immediately after the injection. The placement was randomized so that half of the rats were placed in BAP chamber on Test Day 1 and in the CHP chamber on Test Day 2 and the other half had the opposite sequence. This paradigm mimicked the conditioning CPP session except that neither chow nor bacon was given.

4.3.2 Image Acquisition and Analysis

Immediately after FDG uptake, rats were anesthetized using isoflurane and scanned under a static imaging protocol for 20 min using a ramp filter with cutoff at Nyquist frequency. Prior to acquisition, the left or right lateral tail vein was punctured using a 25g needle and blood-glucose levels were measured with a standard glucometer (Truetrack, CVS) to ensure normal blood glucose. All scans were conducted between 1300 and 1800 hrs. Images were reconstructed using the *maximum-a-posteriori* algorithm (15 iterations, 0.01 smoothing value, 256x256 resolution) with reconstruction voxel sizes corresponding to x=0.42, x y=0.42 x and z=1.21 mm. Images were spatially normalized and coregistered to a magnetic resonance image (MRI) atlas (Thanos et al., 2008b) using PMOD software (PMOD Technologies, Zurich, Switzerland). A custom designed region of interest (ROI) template (regions identified using the Paxinos atlas) was then applied to the MRI atlas. The template comprised the following 24 ROIs: olfactory bulb (OB), prefrontal cortex (PFC), cingulate cortex (CG), motor cortex (M1), orbital cortex (OR), insular cortex (IR), somatosensory cortex (SO), nucleus accumbens (NAc), ventral pallidum (VP), caudate putamen (CPu), dorsal midbrain (DMB), ventral midbrain (VMB), dorsal pons (DPo), ventral pons (VPo), parietal cortex (PtA), temporal cortex (TeA), visual cortex (VC), auditory cortex (AU), hippocampus (HP), thalamus (TH), hypothalamus (HYP), amygdala (AM), retrosplenial cortex (RS), and cerebellum (CB). FDG uptake values were reported in kBq/cc and normalized for injected dose, body weight and blood glucose levels as previously described (Thanos et al., 2008a). To quantify cue-induced changes in BGluM, the difference between bacon and chow cue-induced BGluM was calculated, and this value was then normalized to chow cue-induced BGluM (represented as percent different).

4.4 EtOH Consumption

4.4.1 Apparatus

One week after the imaging experiments two cylindrical polypropylene drinking tubes (150 ml) were filled with either EtOH (2%, 4%, 6%, 8%) or water, fitted with a one-hole rubber stopper and a drinking spout, and placed in each rat's cage. The positions of EtOH and water bottles were switched daily, and contents were also flushed and refilled daily. Volume of EtOH and water consumed (adjusting for spill volume) was measured daily between 1000 and 1200 hrs.

4.4.2 Procedures

Starting on Experiment Day 1, all rats were presented with a 2-bottle choice of one bottle of water, and one bottle of 2% v/v EtOH. After 4 days, the EtOH concentration was increased to 4% v/v for another 4 days, and then 6% for 2 weeks, and finally 8% for 3 days. This gradual habituation method is to habituate rats to 8% EtOH solution and allows for differentiating EtOH intake sensitivity among groups of rats (Gustafsson et al., 2005; Ploj et al., 2003).

4.5 Quantitative Real-Time PCR (qPCR)

4.5.1 Tissue Preparation

Rats were sacrificed one month after the EtOH consumption sessions and their brains removed and frozen. Brains were sectioned (20 µm thickness) at the PFC level using a cryostat (Microm HM560, Thermo Scientific, Rockford, IL) and a dissecting microscope and sterile scalpel were used for tissue collection. Slides were kept on dry ice throughout the tissue isolation procedure. Total RNA was isolated with the RNAGEM Tissue Plus extraction kit (ZyGEM, Hamilton, New Zealand) following this kit's instructions. After RNA extraction, PCR was performed to synthesize cDNA using qScript cDNA Supermix reagent (Quanta BioSciences, Gaithersburg, MD). In brief, each sample's template RNA (5 µL) was mixed with reaction Supermix (4 µL) and PCR-grade water (11 µL) to yield a volume of 20 µL per reaction. Amplification was performed in a MyCycler thermal cycler (Bio Rad, Hercules, CA) set to: (*i*) 25°C for 5 min, (*ii*) 42°C for 30 min and (*iii*) 85°C for 5 min, and then kept at 4°C until collected. This was then diluted 1:2 with PCR-grade water for a final volume of 40 µL. Synthesized cDNA was stored at -30°C and extracted RNA at -80°C.

4.5.2 TaqMan Gene Expression Assays

TaqMan gene expression assays (Applied Biosystems, Carlsbad, CA) were used to quantify mRNA expression of *Cnr1 (Rn00562880_m1), DLG4 (Rn00571479_m1), Drd1a (Rn03062203_s1)*, *Drd2 (Rn01418275_m1)*, *Gria1 (Rn00709588_m1), Gria2 (Rn00568514_m1), Grin1 (Rn01436038_m1), Grin2a (Rn00561341_m1), and Grin2b (Rn00680474_m1)*. All assays were performed in triplicate. Genes were selected *a priori* based on their involvement in synaptic transmission in PFC and association with conditioning and addiction (glutamate, dopamine, endocannabinoids).

4.5.3 Analysis

The *dd*C_t method was used to determine relative mRNA expression. To calculate relative expression, 18S rRNA was simultaneously measured in each well using a VIC-labeled probe (cat number: 4319413E, Applied Biosystems). If either probes' C_t value for a given sample was identified as an outlier, using the Grubb's method, then this animal's values were removed from the analysis. Linear regression analysis was used to examine relationships between mRNA expression, EtOH consumption and BGluM.

4.5.4 Statistics

All values are reported as means (± SEM) unless otherwise noted. T-tests were used to assess significance for behavioral data and ANOVA was used for BGluM. Linear regression analysis was used to assess correlation between the variables examined. For all statistical tests, we used $p < 0.05$. We did not correct for multiple comparisons as there is *a priori* justification for not adjusting the threshold *p* values for each comparison (see discussion).

Acknowledgements

This work was supported by the NIAAA (AA 11034 & AA07574, AA07611). MM was supported by the T32 NIDA Training grant (5T32DA007135) at Mount Sinai School of Medicine.

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Abbreviations

EtOH - ethanol
BAP - bacon-paired
CHP - chow-paired
BGluM - brain glucose metabolism
NMDA- N-methyl-D-aspartic-acid
µPET - small animal positron emission tomography
CPP - conditioned place preference
FDG - 2-fluoro-2-deoxy-D-glucose
IV - intravenous
IP -intraperitoneal
OB - olfactory bulb
CG - cingulate cortex
M1 -motor cortex
OR - orbital cortex
IR - insular cortex
SO - somatosensory cortex
NAc - nucleus accumbens
VP - ventral pallidum
CPu - caudate putamen
DMB - dorsal midbrain
VMB - ventral midbrain
DPo -dorsal pons
VPo -ventral pons
PtA - parietal cortex
TeA - temporal cortex
VC - visual cortex
AU - auditory cortex
HP - hippocampus
TH - thalamus
HYP - hypothalamus
AM - amygdala
RS - restrosplenial cortex
CB - cerebellum

Figures

Figure 1

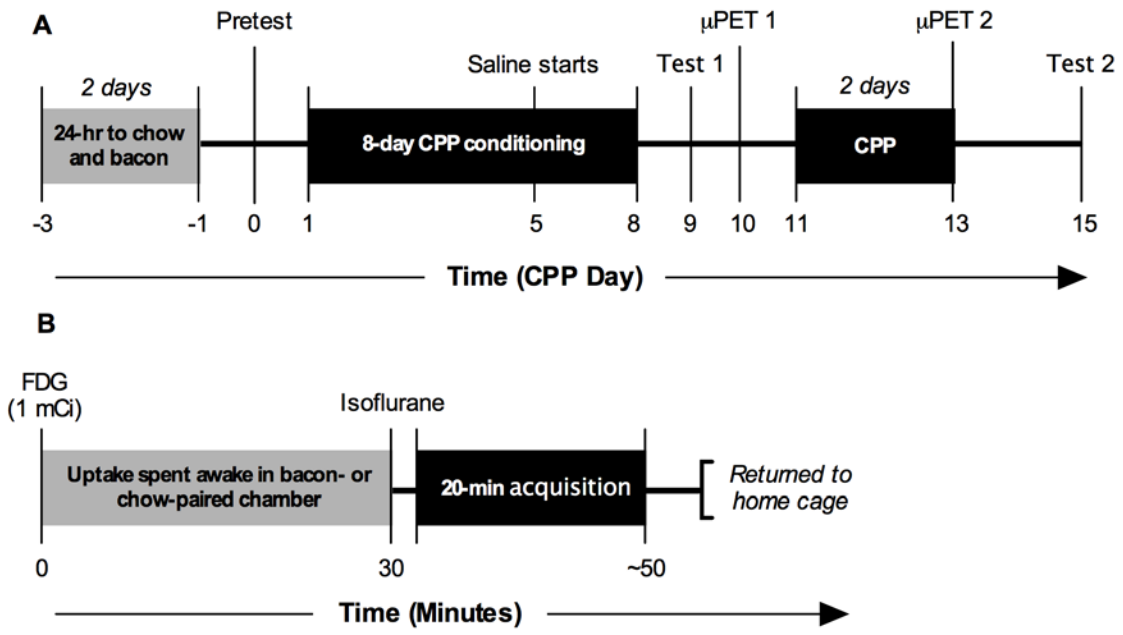

Figure 1. Animals were conditioned to chambers paired with bacon or chow, and then scanned using FDG when exposed to each chamber without the paired food-cue. (A) Animals were conditioned to distinct cues using CPP, assessed for sustained cue-preference (Test 1 and 2) and scanned using FDG-μPET (μPET 1 and 2). To reduce neophobia, animals were initially exposed to bacon, along with chow, twice prior to conditioning (Habituation period, Days 3 through 1). (B) On scan days, animals were injected with FDG, and during the uptake phase (~30 minutes), animals were conscious and placed in either the BAP or CHP chamber. Each animal's BAP and CHP scans occurred on separate days, and the order was random and counter-balanced.

Figure 2.

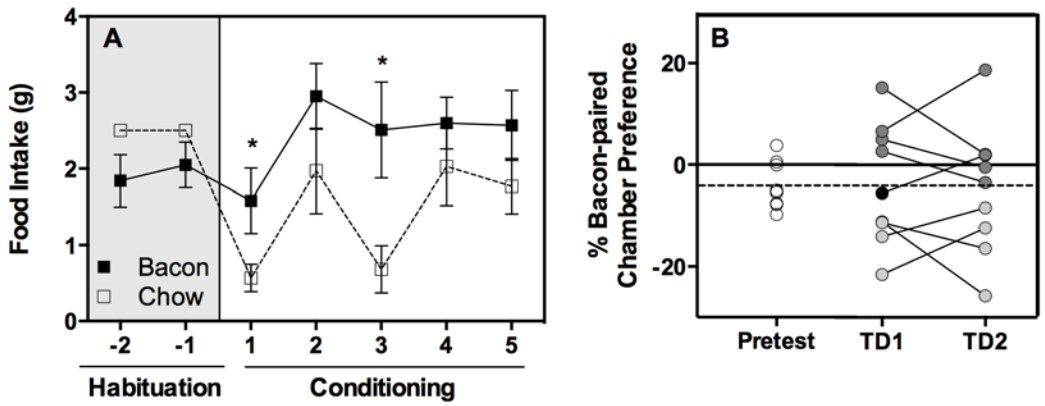

Figure 2. Consumption of appetitive rewards and cue-preference for these rewards. (A) Whereas chow intake was variable, bacon consumption rapidly escalated, then stabilized, and was significantly greater than chow consumption on conditioning Days 1 and 3 (*$p < 0.05$ compared to chow). (B) After 8-days of conditioning, there was no change in the amount of time spent in the bacon-paired (BAP) chamber. Individual points indicate each animal. The dotted line indicates the mean preference on Pretest. Preferences above baseline indicate bacon cue-preference (dark gray), below indicate chow cue-preference (light gray), and a single animal (black) did not show clear preference.

Figure 3.

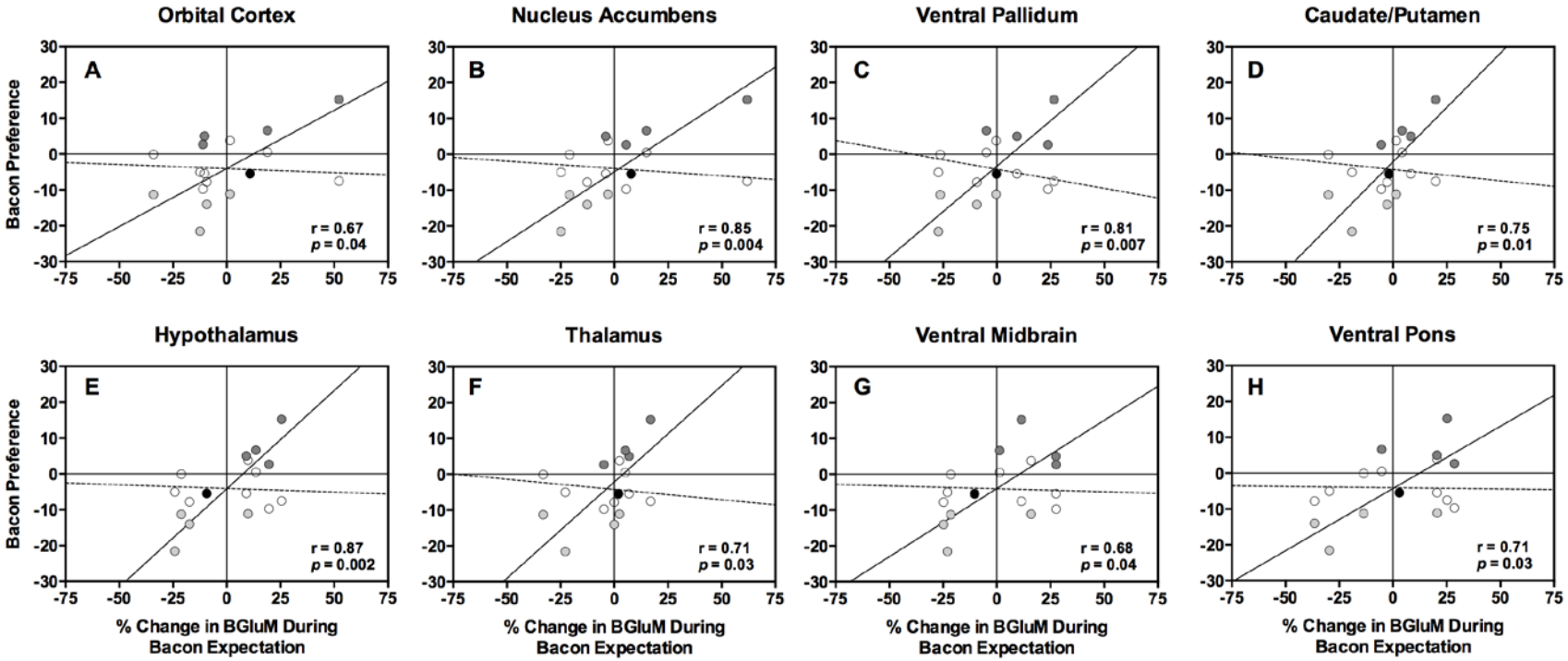

Figure 3. Brain glucose metabolism (BGluM) during bacon expectation was significantly correlated with bacon chamber preference after conditioning but not before conditioning. (A) Orbital Cortex, (B) Nucleus Accumbens, (C) Ventral Pallidum, (D) Caudate/Putamen, (E) Hypothalamus, (F) Thalamus, (G) Ventral Midbrain and (H) Ventral Pons. (Open circles = Pretest; closed circles = Test Day 1, color-coded to indicate preference-strength; dotted line = fit corresponding to pretest; solid line = fit corresponding to Test Day 1.)

Figure 4.

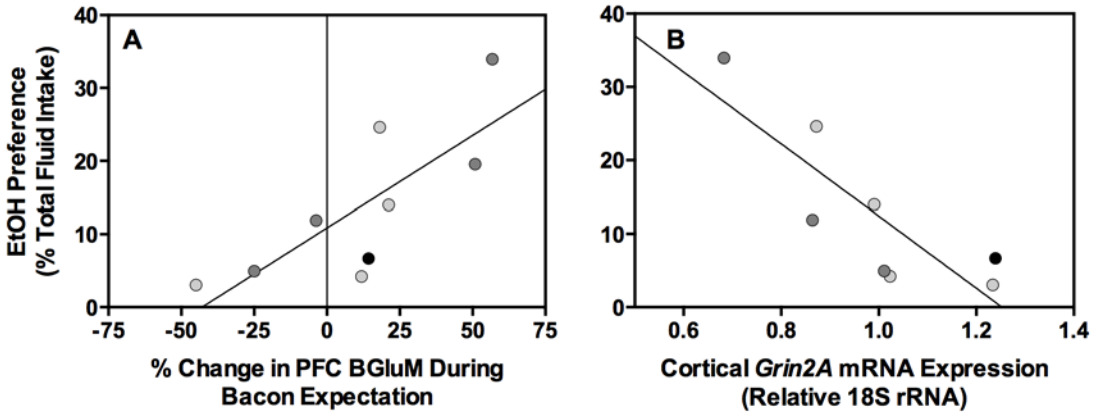

Figure 4. Cortical cue-induced metabolism predicts future preference for an entirely different, yet unfamiliar reward. (A) Regional brain glucose metabolism (BGluM) in response to bacon-paired cues in prefrontal cortex was significantly correlated with 8% ethanol (EtOH) preference. (B) Relative expression of *Grin2A* mRNA in the prefrontal cortex was negatively correlated with EtOH preference. (In both, dark gray indicates bacon cue-preference while light gray indicates chow cue-preference, see Figure 2B for details.)