The Effects of Chronic Alcohol Self-Administration on Serotonin-1A Receptor Binding in Nonhuman Primates

Ansel T. Hillmer[1,2], **Dustin W. Wooten**[1,2], **Dana L. Tudorascu**[3,4], **Todd E. Barnhart**[2], **Elizabeth O. Ahlers**[1], **Leslie M. Resch**[5,6], **Julie A. Larson**[5,6], **Alexander K. Converse**[1], **Colleen F. Moore**[7,8], **Mary L. Schneider**[5,6,7], and **Bradley T. Christian**[1,2,9]

[1]Waisman Laboratory for Brain Imaging and Behavior, University of Wisconsin, Madison

[2]Department of Medical Physics, University of Wisconsin, Madison

[3]Department of Internal Medicine, University of Pittsburgh

[4]Department of Biostatistics, University of Pittsburgh

[5]Harlow Center for Biological Psychology, University of Wisconsin, Madison

[6]Department of Kinesiology, University of Wisconsin, Madison

[7]Department of Psychology, University of Wisconsin, Madison

[8]Department of Psychology, Montana State University

[9]Department of Psychiatry, University of Wisconsin, Madison

Abstract

Background—Previous studies have found interrelationships between the serotonin system and alcohol self-administration. The goal of this work was to directly observe *in vivo* effects of chronic ethanol self-administration on serotonin 5-HT$_{1A}$ receptor binding with [^{18}F]mefway PET neuroimaging in rhesus monkeys. Subjects were first imaged alcohol-naïve and again during chronic ethanol self-administration to quantify changes in 5-HT$_{1A}$ receptor binding.

Methods—Fourteen rhesus monkey subjects (10.7-12.8 years) underwent baseline [^{18}F]mefway PET scans prior to alcohol exposure. Subjects then drank gradually increasing ethanol doses over four months as an induction period, immediately followed by at least nine months *ad libidum*

Corresponding Author: Ansel Hillmer 2 Church St, Suite 511 New Haven, CT 06511 ansel.hillmer@yale.edu.

ethanol access. A post [^{18}F]mefway PET scan was acquired during the final three months of *ad libidum* ethanol self-administration. 5-HT$_{1A}$ receptor binding was assayed with binding potential (BP_{ND}) using the cerebellum as a reference region. Changes in 5-HT$_{1A}$ binding during chronic ethanol self-administration were examined. Relationships of binding metrics with daily ethanol self-administration were also assessed.

Results—Widespread increases in 5-HT$_{1A}$ binding were observed during chronic ethanol self-administration, independent of the amount of ethanol consumed. A positive correlation between 5-HT$_{1A}$ binding in the raphe nuclei and average daily ethanol self-administration was also observed, indicating that baseline 5-HT$_{1A}$ binding in this region predicted drinking levels.

Conclusions—The increase in 5-HT$_{1A}$ binding levels during chronic ethanol self-administration demonstrates an important modulation of the serotonin system due to chronic alcohol exposure. Furthermore, the correlation between 5-HT$_{1A}$ binding in the raphe nuclei and daily ethanol self-administration indicates a relationship between the serotonin system and alcohol self-administration.

Keywords

Serotonin; 5-HT$_{1A}$; PET Imaging; Chronic Alcohol Self-Administration; Rhesus Monkey

1. INTRODUCTION

The 5-hydroxytryptamine (5-HT), or serotonin, system is a crucial neurotransmitter system that regulates learning, mood, and anxiety. Dysfunction of this system has been implicated in many neuropsychiatric illnesses including depression, schizophrenia, and other disorders relating to mood and anxiety (Jans et al., 2007). There is also extensive evidence linking alcohol-related disorders with the serotonin system (see LeMarquand et al., 1994; Sari et al., 2011 for reviews). In particular, Type II alcoholism, which is characterized by impaired impulse control, has been heavily connected with deficits in serotonergic functioning (Cloninger, 1987). Low levels of 5-hydroxindoleacetic acid (5-HIAA), the primary metabolite of 5-HT, have been observed in the CSF of Type II alcoholics (Virkkunen and Linnoila, 1993; Linnoila et al., 1994). Additionally, studies examining the serotonin transporter (5-HTT) found reduced 5-HTT density in post-mortem alcoholics (Mantere et al., 2002).

Serotonin 5-HT$_{1A}$ receptors occur both as autoreceptors in the raphe nuclei that regulate 5-HT tone (Blier et al., 2003), and postsynaptically on inhibitory neurons throughout the rest of the brain (Raymond et al., 1999). The critical role these 5-HT$_{1A}$ receptors play in regulating 5-HT transmission makes them an important target for understanding interactions of ethanol with the serotonin system (Sari et al., 2011). Moreover, 5-HT$_{1A}$ receptors are heavily implicated in anxiety and depression, making them particularly vital targets for studies investigating behavioral traits associated with alcoholism.

Neuroimaging studies in humans have provided important in vivo data concerning alcohol"s effects on the serotonin system, though the findings are mixed. One study explicitly examined 5-HT$_{1A}$ receptors in addition to 5-HTT binding, and found no difference in

binding levels between alcoholic patients and healthy controls for both serotonergic targets (Martinez et al., 2009). Further experiments examining the 5-HTT yielded varying results, with one study finding no difference in 5-HTT availability in alcoholic patients relative to control subjects (Brown et al., 2007), while earlier investigations found lower 5-HTT binding in alcoholics compared to healthy controls (Heinz et al., 1998; Szabo et al., 2004). Striatal $5\text{-}HT_{1B}$ levels were also significantly increased in alcoholics (Hu et al., 2010).

The previously referenced neuroimaging experiments examined *correlational* relationships between the serotonin system and ethanol exposure, and are demonstrative of the majority of studies designed to link 5-HT dysfunction with alcohol exposure. However, there is a lack of experiments directly examining *causal* effects of ethanol on serotonin function, particularly in primate species. In rats, microdialysis and in vitro studies have shown synaptic serotonin to decrease during a chronic presence of ethanol (Kirby et al., 2011).

The use of positron emission tomography (PET) imaging offers a unique opportunity for causal experiments directly assaying receptor binding during chronic ethanol exposure in primate species. Studies of nonhuman primates combine the social and behavioral similarities between nonhuman primates and humans with advantages of greater control over experimental variables in a laboratory setting, including precise knowledge of a subject"s ethanol exposure history, data from adult subjects in an ethanol-naive state, and control over abstinence during scanning procedures (Barr and Goldman, 2006).

The goal of the present work was to use PET imaging with the $5\text{-}HT_{1A}$ specific radioligand $[^{18}F]$mefway to assay $5\text{-}HT_{1A}$ binding before and during chronic ethanol self-administration in rhesus monkeys, allowing for direct measures of changes in $5\text{-}HT_{1A}$ binding due to an extended presence of ethanol. Secondary analyses were conducted to assess the relationship between $5\text{-}HT_{1A}$ availability and ethanol consumed *ad libidum.* These studies yield crucial insight into the modifications chronic ethanol exposure impose on serotonin $5\text{-}HT_{1A}$ receptors.

2. METHODS

2.1. Subjects

The experimental cohort consisted of fourteen rhesus monkeys (*Macaca mulatta*; 7 males, 7 females), drawn from an experimental protocol designed to examine the effects of gestational timing of prenatal ethanol exposure on development (for details, see Schneider et al., 2001). At the start of these experiments, no post-natal alcohol exposure was experienced by any subjects. All housing and experimental guidelines were approved by the Institutional Animal Care and Use Committee. These procedures follow the guidelines addressing the ethical care and use of laboratory animals as set forth by the USDA "Federal Register" standards and the "Guide for the Care and Use of Laboratory Animals" set forth by the NIH (Bethesda, MD, USA).

2.2. Experimental Procedures

To summarize the experimental protocol, shown in Figure 1, subjects underwent a "baseline" PET scan quantifying $5\text{-}HT_{1A}$ binding prior to chronic ethanol drinking. Age at

the time of the first PET scan was 10.7-12.8 years. Subjects then self-administered (drank) ethanol over a 390 day (13 month) period, adapting procedures developed previously (Vivian et al., 2001). The first 120 days (4 months) consisted of a controlled ethanol dose induction period, while the final 270 days (9 months) allowed subjects to consume ethanol ad-libidum. During the final 90 days of ethanol exposure, a "post" PET scan was acquired to examine 5-HT$_{1A}$ binding levels during chronic alcohol self-administration.

2.2.1. Ethanol Drinking Protocol—An operant drinking panel, described in detail by Vivian and colleagues (2001), controlled and monitored subject access to food pellets, water, and ethanol solution. For every phase of the study, alcohol was available as 4% v/v ethanol water solution.

The first 120 days (4 months) of ethanol access consisted of an induction period, where subjects self-administered (drank) controlled, gradually increasing ethanol doses using scheduled-induced polydipsia. During this induction period, food pellets were regularly delivered every 180-300 seconds during a 16-hour session every day. The fixed time interval was designed to encourage animals to drink a targeted alcohol dose. To avoid conditioned taste aversion to ethanol, the induced ethanol self-administration dose was escalated in a step-wise fashion. Thus, subjects consumed only water for the first 30 days, followed by 0.5 g/kg/day ethanol for the second 30 days, 1.0 g/kg/day ethanol for the third 30 days, and 1.5 g/kg/day ethanol for the final 30 days of induction.

Immediately following ethanol induction, subjects were allowed to drink ethanol *ad libidum* for 270 days (9 months). During the *ad libidum* period, ethanol/water solution (4% v/v) and water were continuously available over a 22-hour session every day. Food was given as 3 meals available every 2 hours over the 22-hour session. Ethanol intake was quantified as average ethanol consumed per day (g/kg/day). Following the *ad libidum* period, ethanol access was terminated, and subjects were monitored for symptoms of withdrawal including nausea, vomiting, and dehydration. Animal weight was also monitored.

The average daily ethanol self-administration for each subject was quantified over the first 6 months of the *ad-libitum* period. Across all subjects, the average daily ethanol consumed was 0.93 ± 0.45 g/kg/day. No withdrawal symptoms were observed following the termination of ethanol access.

2.2.2. PET Scanning Sessions—Measures of 5-HT$_{1A}$ receptor binding were assayed with [^{18}F]mefway PET imaging sessions. The PET radioligand [^{18}F]mefway is highly specific to 5-HT$_{1A}$ receptors with well-validated kinetic properties in the rhesus monkey (Wooten et al., 2011). Radiotracer was produced following previously reported methods (Saigal et al., 2006), yielding high specific activity [^{18}F]mefway (>70 GBq/μmol at injection). For post [^{18}F]mefway scans, ethanol access was terminated approximately 90 minutes prior to scan initiation to minimize acute abstinence effects from influencing measured 5-HT$_{1A}$ binding levels. Since alcohol was self-administered, the resulting time between last drink and scan start ranged from two to 14 hours.

On the day of scans, subjects were anesthetized one hour prior to imaging with 10 mg/kg IM ketamine. Anesthesia was induced at 1.5% isoflurane with 2 L of oxygen and then maintained at 1.0-1.5% isoflurane with 2 L of oxygen throughout the imaging session. All vital signs, including body temperature, respiration rate, heart rate, SpO_2 levels, and capnography were monitored throughout the duration of scans. A microPET P4 scanner was used for image data acquisition (Tai et al., 2001). A 518 s ^{57}Co transmission scan was first acquired to correct for the scatter and attenuation of radiation in the tissue. A 30 s bolus injection of 59-130 MBq high specific activity [^{18}F]mefway then initiated PET data acquisition, which lasted for 90 minutes. Following study completion, anesthesia was terminated and subjects were returned to their cages and monitored until fully alert.

2.3. PET data processing

Dynamic PET data were histogrammed into time bins up to 5 minutes long and reconstructed with filtered back projection (0.5 cm^{-1} ramp filter). Corrections for scanner normalization, dead time, signal attenuation, radioactive decay, and scatter were included. Final reconstructed PET images had a matrix size of 128×128×63 with spatial dimensions of 1.90×1.90×1.21 mm^3.

[^{18}F]Mefway binding was quantified by binding potential relative to nondisplaceable uptake (BP_{ND}). BP_{ND} is proportional to 5-HT$_{1A}$ receptor sites available for radioligand binding (*Bavail)* and [^{18}F]mefway"s apparent affinity for these sites (*1/K$_D$*) (Innis et al., 2007). The mulitilinear reference tissue model (MRTM2; Ichise et al., 2003) was used with the cerebellum as a reference region (Wooten et al., 2011) to produce voxel-wise BP_{ND} parametric maps. Our previous experience using [^{18}F]mefway has yielded a BP_{ND} test-retest variability of 8-11% (unpublished data).

Since individual subject MRIs were not acquired, a template based approach was used for region of interest (ROI) definition. Parametric maps were transformed to a standardized PET space using an in-house [^{18}F]mefway template coregistered to a rhesus monkey MRI atlas (McLaren et al., 2009). The optimal transformation matrix was determined with FSL-FLIRT (Jenkinson et al., 2002), allowing for 7 degrees of freedom. The spatially normalized images were then smoothed with a 4×4×4 mm^3 Gaussian filter to reduce anatomical differences between subjects in the images.

ROIs were selected for analysis based on brain areas of [^{18}F]mefway specific binding with similar kinetic properties and physiological significance implemented previously by our lab (Christian et al., 2013). Regions extracted with a digital rhesus monkey template included anterior cingulate gyrus (aCg), posterior cingulate gyrus (pCg), hippocampus (Hp), amygdala (Am), dorsolateral prefrontal cortex (pFC), parietal cortex (PC), occipital cortex (OC), and lateral temporal cortex (lTC). Since the raphe nuclei region (RN), which consisted of a small volume containing high 5-HT$_{1A}$ density, exhibited variability in location despite spatial normalization, the RN was manually defined on the normalized images with a 3 voxel diameter circle drawn on 3 consecutive transaxial planes.

2.4. Statistical Analysis

Analysis was carried out to test for differences in baseline BP_{ND} and post BP_{ND}. Paired t-tests were used to test the hypothesis of no difference between the means of baseline BP_{ND} compared to post BP_{ND} for each region. The resulting p-values from these hypotheses indicating differences between baseline BP_{ND} and post BP_{ND} were corrected for multiple comparisons due to the examination of multiple regions. The False Discovery Rate correction with $\alpha < 0.05$ was implemented for the multiple comparison correction (Benjamini et al., 1995). The corresponding adjusted confidence intervals are also reported (Benjamini and Yekutieli, 2005). Effect sizes for differences between baseline BP_{ND} and post BP_{ND} were computed with Cohen"s d effect sizes (Cohen, 1988).

Univariate analysis was used to test for correlations between binding measures and ethanol self-administration. Pearson"s correlation coefficients between average daily ethanol consumed (g/kg/day) over the *ad libidum* drinking period and baseline BP_{ND}, post BP_{ND}, and change in BP_{ND} were calculated. The 95% confidence interval was computed for the Pearson"s correlation using Fisher"s z transformation. The correlation coefficient"s p-values and their confidence interval levels were not corrected for multiple hypothesis tests. Analysis was performed using SAS (version 9.3, SAS Ipearnstitute, Cary NC) and R (version 3.0) statistical software.

3. RESULTS

Statistically significant ($\alpha < 0.05$) increases in mean 5-HT$_{1A}$ binding ([^{18}F]mefway BP_{ND}) following chronic ethanol self-administration were observed in all regions except for the RN, shown in Figure 2. The p-value in the RN approached but did not reach statistical significance (p=0.059). The average change in BP_{ND} for all regions was 0.34 (16%) with a standard deviation of 0.13. The average effect size for all the regions was 0.59, which is regarded as a medium-high effect size (Cohen, 1988). These data are presented for each region in Table 1, featuring observed differences between baseline and post BP_{ND} with adjusted 95% confidence intervals, p-values (both uncorrected and corrected), and Cohen"s d effect sizes.

A second major finding was a positive association between baseline BP_{ND} and average daily ethanol self-administration in the RN (r=0.609; p=0.021; 95% CI (0.12, 0.86)), shown in Figure 3A. Similarly, a significant positive association was found between post BP_{ND} and average daily ethanol self-administration in RN (r=0.595; p=0.024; 95% CI (0.09, 0.86)), shown in Figure 3B. No other regions exhibited significant relationships between baseline or post BP_{ND} and daily ethanol self-administration. No significant relationships between change in BP_{ND} and daily ethanol self-administration were observed. Table 2 summarizes these results, including the Pearson"s r and p-values calculated in each region for each BP_{ND} measure (baseline, post, and change in BP_{ND}) correlated with daily average ethanol self-administration.

4. DISCUSSION

This study presents two important findings relating *in vivo* 5-HT$_{1A}$ binding levels with chronic ethanol self-administration. First, 5-HT$_{1A}$ binding levels increased during chronic ethanol self-administration in the rhesus monkey. This finding is consistent with either increased 5-HT$_{1A}$ receptor density, decreased synaptic 5-HT concentrations, or a combination of the two effects. Second, 5-HT$_{1A}$ binding in the RN both before and during ethanol self-administration positively correlated with average daily ethanol self-administration. This provides evidence for altered 5-HT function influencing ethanol self-administration. The present experiments fill a crucial gap directly examining causal modifications to 5-HT$_{1A}$ receptor binding in vivo following chronic ethanol self-administration in primate species.

4.1. Interpretation of Results

Precise interpretation of the primary finding, increases to 5-HT$_{1A}$ BP_{ND} during chronic ethanol self-administration, is complicated by the multiple influences on radioligand binding at receptor sites. Decreased extracellular 5-HT, as observed after prolonged ethanol exposure in microdialysis studies (Bare et al., 1998; Thielen et al., 2004), would reduce synaptic 5-HT competition at the 5-HT$_{1A}$ site, increasing [^{18}F]mefway BP_{ND} measured with PET procedures. Alternatively, increases in receptor density (B_{max}) would also increase BP_{ND}. Recent post mortem studies demonstrated increased hippocampal 5-HT$_{1A}$ B_{max} for monkeys self-administering alcohol relative to healthy controls in cynomolgus macaques (Burnett et al., 2014). Notably, those subjects underwent a similar ethanol drinking paradigm to the present studies. The increase in 5-HT$_{1A}$ binding during chronic ethanol self-administration could speculatively be a combination of the two effects, where lowered synaptic 5-HT induced by chronic ethanol exposure upregulates 5-HT$_{1A}$ receptors as a compensatory mechanism. The likely increase in 5-HT$_{1A}$ receptor density represents, to our knowledge, a first reported direct *in vivo* observation of modulations to 5-HT$_{1A}$ receptors induced by chronic alcohol exposure in primate species.

A secondary result of the present work was a positive relationship between average daily *ad libidum* ethanol self-administration and 5-HT$_{1A}$ binding in the RN both at baseline and post ethanol drinking. The RN is a crucial component of the serotonin system because these 5-HT$_{1A}$ receptors function as autoreceptors to regulate synaptic 5-HT tone (Raymond et al., 1999; Blier et al., 2003). Furthermore, the RN is the source of dense serotonergic projections to cortico-midbrain regions (Johnson, 2004). Therefore, we interpret these findings to suggest that fundamental differences in 5-HT function may underlie intrasubject variation in ethanol self-administration.

Interestingly, for all regions no relationship between the change in 5-HT$_{1A}$ BP_{ND} (between baseline and post-drinking) and average daily ethanol self-administered was observed. Considering the observed global increases in binding during chronic ethanol self-administration, we speculate that a prolonged presence of ethanol induces a uniform response from the 5-HT system throughout the brain. The large inter-subject range for 5-HT$_{1A}$ RN BP_{ND} binding (approximately 100%) observed between the lowest and highest ethanol consumers makes it unlikely that our measures lacked the sensitivity to identify

subtle changes in 5-HT_{1A} binding. These data therefore provide crucial cohesion between the primary findings of this work by suggesting that the 5-HT involvement (via the RN) in predicted average daily ethanol self-administration is independent from the mechanistic global increases in 5-HT_{1A} binding during chronic ethanol consumption.

4.2. Further Statistical Considerations and Limitations

The primary analysis of differences in BP_{ND} baseline and during drinking yielded a non-significant yet strong trend (p=0.059) of increased binding in RN. Considering the increased variability in PET measurements due to the small volume of this region (>400% smaller than any other region), we therefore speculate that the RN is consistent with the other regions in increased 5-HT_{1A} binding during chronic ethanol self-administration.

Our group has previously reported significant relationships between [^{18}F]mefway BP_{ND} and both 5-HTTLPR genotype (Christian et al., 2013) and subject sex (Wooten et al., 2013). These factors did not influence the observed increases in 5-HT_{1A} binding following chronic ethanol exposure since each subject serves as its own control. For the correlation analyses, two-sample t-tests found no statistically significant differences in BP_{ND} between 5-HTTLPR genotypes or subject gender due to the small sample size, justifying the use of a univariate statistical model.

The present subjects were part of a cohort of animals whose mothers were exposed to alcohol while subjects were *in utero* (Schneider et al., 2001), with subjects drawn from all treatment groups. There was no evidence for any trends between treatment groups and 5-HT_{1A} binding levels. Consequently, prenatal treatment group was not included as a covariate in the analysis, particularly since the small group sizes yielded poor statistical power. The potential confound of pre-natal alcohol exposure should be considered in interpretation of correlations between 5-HT_{1A} binding in RN and average daily alcohol self-administration, as this may have conceivably influenced the serotonin system at baseline or alcohol self-administration (or both).

Sensitivity analysis suggested that the potential presence of ethanol during post PET scans also did not influence the two main findings. Four subjects consumed ethanol within four hours of their post PET scan, and thus potentially had ethanol in their system during PET procedures, while the other ten did not consume alcohol for at least eight hours prior to PET scans. For the sensitivity analysis, the data were re-analyzed excluding the four subjects who consumed ethanol within four hours of their post PET scan. Statistical significance, including the false discovery rate correction, was maintained for the primary finding of increased 5-HT_{1A} binding across most brain regions, and a similar correlation coefficient was observed between RN post BP_{ND} and average daily ethanol self-administration (*r*=0.545). Previous microdialysis studies found that prior exposure to ethanol dampened the release of synaptic 5-HT following subsequent ethanol exposure (Bare et al., 1998), suggesting that the presence of ethanol during PET scans minimally influences radioligand binding levels in a chronic ethanol exposure paradigm.

4.3. Implications of Results

The positive relationship between RN 5-HT$_{1A}$ binding and ethanol self-administration exhibits striking similarities to studies examining CSF 5-HIAA concentrations in connection with ethanol exposure. In humans, 5-HIAA levels are lower in Type II alcoholics (Linnoila et al., 1994), and these findings have been extensively replicated in rhesus monkeys (Higley et al., 1996). Notably, in rhesus monkeys a negative correlation between 5-HIAA levels and ethanol self-administration was observed. Furthermore, inter-individual 5-HIAA levels were shown to be stable over time (Higley et al., 1992), providing evidence that low 5-HIAA levels may be an enduring trait of 5-HT deficit connected to increased ethanol self-administration. In our findings, the positive correlation between 5-HT$_{1A}$ binding in the RN and daily ethanol self-administration is notably present both in an alcohol-naive state and following ethanol exposure, suggesting that increased RN 5-HT$_{1A}$ binding may be an alternative manifestation of reduced 5-HIAA and 5-HT deficit present in increased ethanol self-administration in rhesus monkeys. In support of this interpretation, a linear model correlating RN post BP$_{ND}$ with daily alcohol self-administration while controlling for RN pre BP$_{ND}$ yielded a weak relationship (r=0.35). This provides additional evidence that 5-HT$_{1A}$ binding in the RN is trait-like, echoing findings examining 5-HIAA levels in nonhuman primates exposed to alcohol (Higley et al., 1996). These findings may potentially be extrapolated to human alcohol patients (e.g., type II alcoholism), but require further human studies to validate this conjecture.

The present results demonstrate that the use of PET imaging of 5-HT$_{1A}$ receptors can be an alternative tool to assess 5-HT deficits associated with increased ethanol self-administration. Sophisticated experimental techniques offer the opportunity to extend these findings by teasing apart differences in receptor upregulation from changes to synaptic serotonin concentrations (Wooten et al., 2012). Additionally, 5-HT$_{1A}$ autoreceptors in the RN may be a target of localized treatment for potential therapeutics in alcohol-related disorders.

Earlier experiments with a PET radioligand analog of [^{18}F]mefway, [^{11}C]WAY-100635, found no difference in 5-HT$_{1A}$ receptor binding levels between alcoholic patients and healthy controls (Martinez et al., 2009). That study, however, tested subjects following 14 days of detoxification. The experiment here minimally withheld ethanol access prior to imaging, thus the duration of ethanol abstinence may speculatively be a contributing factor to the different findings. In vitro microdialysis studies demonstrated an effect of ethanol abstinence duration on the serotonin system, as 35% lower extracellular 5-HT concentrations were observed in rats continually exposed to ethanol compared to controls, while this effect was not observed following 2 weeks ethanol deprivation (Thielen et al., 2004). The opportunity to minimize ethanol abstinence in rhesus monkeys thus offered a major advantage over clinical protocols in that the present experiments directly examined causal effects of chronic ethanol self-administration on serotonin function. No follow-up scans were performed on the present cohort after extended alcohol abstinence. The extent to which 5-HT$_{1A}$ binding levels may recover after alcohol abstinence remains an important question for future experiments.

Previous neuroimaging studies of the serotonin transporter (5-HTT) found decreased 5-HTT binding levels in the midbrain (interpreted as the raphe nuclei) of alcoholics compared to

healthy controls (Heinz et al., 1998; Szabo et al., 2004). Later studies using [^{11}C]DASB, a radioligand more specific to 5-HTT, found no difference in binding between alcoholic patients and healthy controls (Brown et al., 2007; Martinez et al., 2009). Notably, [^{11}C]DASB exhibits improved kinetic properties over the radioligands used previously (Szabo et al., 2002). An earlier neuroimaging study examining 5-HT receptors with [^{11} 1B C]P943 revealed increased binding in the 5-HT$_{1B}$ dense region of ventral striatum in alcoholic subjects relative to healthy controls (Hu et al., 2010), demonstrating a difference in the 5-HT system between alcoholics and healthy controls. Future longitudinal studies are needed to examine the causal effects of chronic ethanol exposure on other components of the 5-HT system.

The nonhuman primate model of ethanol self-administration used herein is a well validated tool for examining ethanol self-administration with many parallels to human alcohol use (Vivian et al., 2001; Grant et al., 2008). In this particular cohort, neither excessive amounts of ethanol self-administration nor withdrawal symptoms were observed, indicating that this study most parallels moderate alcohol drinking in humans. These experiments are crucial complements to studies of alcoholic patients in human subjects to examine causal effects of moderate alcohol drinking on neurotransmitter systems with PET imaging. Similar studies in a different cohort have demonstrated an increase in α4β2 nicotinic acetylcholine receptor binding in the cortex following chronic alcohol self-administration (Hillmer et al., 2014). Future studies are underway to exploit these advantages to further study the serotonergic system and the dopaminergic system during moderate ethanol exposure, and include behavior correlates into analyses of these data.

4.4 Conclusion

To conclude, increases in 5-HT$_{1A}$-specific binding were observed following chronic ethanol self-administration in rhesus monkeys. A positive relationship between 5-HT$_{1A}$ binding (both pre- and post- chronic ethanol self-administration) and daily *ad libidum* ethanol consumed was also observed in the raphe nuclei. These findings provide crucial evidence for the modification of serotonin system by the chronic presence of ethanol, and demonstrate the utility of PET imaging in assessing abnormal 5-HT$_{1A}$ function in alcohol-related disorders.

Acknowledgments

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Highlights

- Rhesus monkeys chronically self-administered (drank) alcohol over 12 months

- PET 5-HT$_{1A}$ binding was measured before and after 9-12 months alcohol drinking

- Global increases in 5-HT$_{1A}$ binding were observed during chronic alcohol drinking

- Raphe Nuclei 5-HT$_{1A}$ binding positively correlated with average alcohol drinking
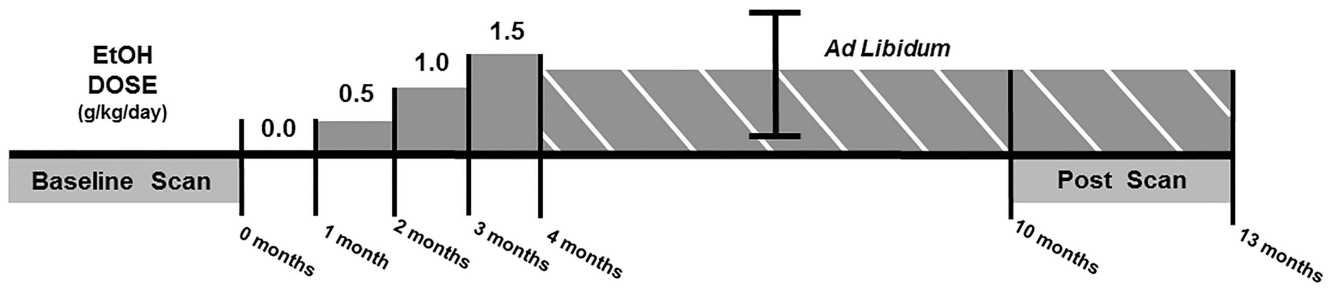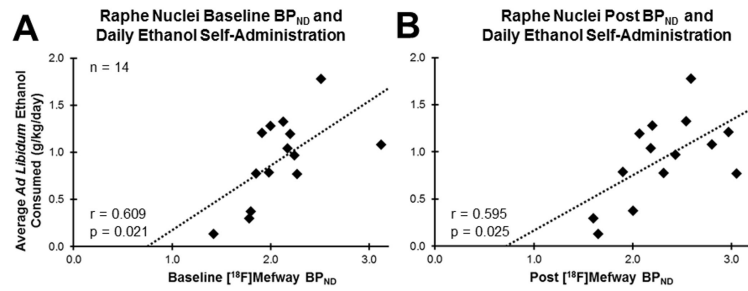
Figure 1.
Alcohol administration protocol during these experiments. Methods are based on procedures developed by Vivian et al., 2001. The x-axis indicates time. Above the y-axis, the ethanol dose is scaled in g/kg/day, while below, periods of PET scanning are indicated.
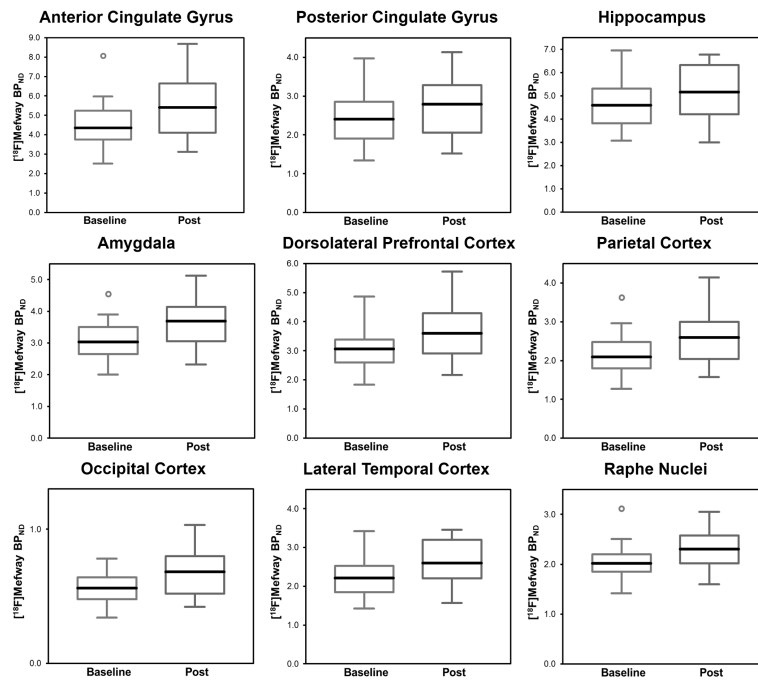
Figure 2.
Boxplots of 5-HT$_{1A}$ BP$_{ND}$ in the regions of interest. Left, in blue, shows baseline BP$_{ND}$, while right, in red, shows post-drinking BP$_{ND}$. ○ indicates outliers. Statistically significant differences were observed in all regions except the raphe nuclei, where a trend (P=0.059) was observed. Note the different scales for each panel. Solid center lines indicate the medians. Each boxplot was composed of data from 14 subjects.

Figure 3.
Relationship between 5-HT$_{1A}$ *BP$_{ND}$* in raphe nuclei and ethanol-self administration (g/kg/day). Panel **A** shows baseline *BP$_{ND}$* while panel **B** shows post *BP$_{ND}$*. Pearson"s correlation coefficients (r) and correlation significance (p) are shown in each panel, bottom left, along with the line fit (dashed line).

Table 1

5-HT$_{1A}$ binding (BP$_{ND}$) in all regions for baseline and post-alcohol.

Region	Baseline BP$_{ND}$	Post BP$_{ND}$	Estimate of BP$_{ND}$ Difference (Adjusted 95% C.I.)	Test statistic	p-value (corrected)	Effect Size (Cohen's d)
Anterior Cingulate Gyrus	3.25±0.95	3.87±1.14	0.61 (0.03,1.19)	t(13)=2.41	.031 (.035)	0.58
Posterior Cingulate Gyrus	1.77±0.52	2.09±0.60	0.32 (0.02, 0.61)	t(13)=2.44	.030 (.035)	0.57
Hippocampus	2.51±0.65	2.89±0.69	0.38 (0.04,0.73)	t(13)=2.55	.024 (.035)	0.57
Amygdala	2.51±0.56	2.93±0.61	0.42 (0.09,0.74)	t(13)=2.92	.011 (.035)	0.71
Dorsolateral Prefrontal Cortex	2.50±0.67	2.95±1.02	0.44 (0.05,0.84)	t(13)=2.58	.023 (.035)	0.51
Parietal Cortex	1.72±0.53	2.05±0.61	0.32 (0.05,0.60)	t(13)=2.69	.018 (.035)	0.57
Occipital Cortex	0.60±0.16	0.73±0.21	0.13 (0.03,0.22)	t(13)=3.04	.009 (.035)	0.68
Lateral Temporal Cortex	1.84±0.46	2.16±0.52	0.32 (0.06,0.59)	t(13)=2.78	.015 (.035)	0.66
Raphe Nuclei	2.10±0.34	2.31±0.45	0.21 (−0.02,0.44)	t(13)=2.07	.059 (.059)	0.49

Table 2

Correlations between average daily ethanol self-administered and binding measurements.

Region	Average Daily Ethanol (g/kg/day) vs. Baseline BP_{ND}		Average Daily Ethanol (g/kg/day) vs. Post BPND		Average Daily Ethanol (g/kg/day) vs. Change in BP_{ND}	
	Pearson's r	p- value	Pearson's r	p- value	Pearson's r	p- value
Anterior Cingulate Gyrus	0.40	0.155	0.32	0.276	0.02	0.933
Posterior Cingulate Gyrus	0.40	0.157	0.32	0.264	0.03	0.911
Hippocampus	0.44	0.118	0.42	0.131	−0.02	0.957
Amygdala	0.38	0.183	0.39	0.178	−0.04	0.890
Dorsolateral Prefrontal Cortex	0.31	0.276	0.28	0.335	−0.02	0.939
Parietal Cortex	0.32	0.268	0.25	0.393	0.04	0.904
Occipital Cortex	0.13	0.648	0.11	0.71	0.00	0.994
Lateral Temporal Cortex	0.36	0.205	0.39	0.178	−0.07	0.805
Raphe Nuclei	*0.61*	*0.021*	*0.60*	*0.023*	−0.07	0.807

The column on far left indicates the region examined. The major columns indicate correlation analyses examining for relationships between daily alcohol self-administration and baseline BP_{ND} (left), post BP_{ND} (middle), and change in BPND (right). Within each main column, sub-columns containing Pearson's correlation coefficients (r) are given on the left, with the corresponding p-value (uncorrected for multiple comparisons) on the right.