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The effects of alcohol on the nonhuman primate brain: A network science approach to neuroimaging

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Abstract

Background—Animal studies have long been an important tool for basic research as they offer a degree of control often lacking in clinical studies. Of particular value is the use of nonhuman primates (NHPs) for neuroimaging studies. Currently, studies have been published using functional magnetic resonance imaging (fMRI) to understand the default mode network in the NHP brain. Network science provides an alternative approach to neuroimaging allowing for evaluation of whole-brain connectivity. In this study we used network science to build NHP brain networks from fMRI data to understand the basic functional organization of the NHP brain. We also explored how the brain network is affected following an acute ethanol pharmacological challenge.

Methods—Baseline resting state fMRI was acquired in an adult male rhesus macaque ($n=1$) and a cohort of vervet monkeys ($n=10$). A follow-up scan was conducted in the rhesus macaque to assess network variability and to assess the effects of an acute ethanol challenge on the brain network.

Results—The most connected regions in the resting state networks were similar across species and matched regions identified as the default mode network in previous NHP fMRI studies. Under an acute ethanol challenge, the functional organization of the brain was significantly impacted.

Conclusion—Network science offers a great opportunity to understand the brain as a complex system and how pharmacological conditions can affect the system globally. These models are sensitive to changes in the brain and may prove to be a valuable tool in long-term studies on alcohol exposure.

Introduction

For over 20 years functional magnetic resonance imaging (fMRI) has played an integral role in understanding hemodynamics in the brain. In the seminal studies of Ogawa et al., the use of the blood oxygen level-dependent (BOLD) signal helped to propel the field of neuroimaging in humans (Ogawa and Lee, 1990, Ogawa et al., 1990). The field was further driven by the introduction of functional connectivity in fMRI to analyze the coherence of signals in the brain (Biswal et al., 1995, Biswal et al., 1997, Lowe et al., 1998). Further

research in the field established a resting state network called the default mode network (Raichle et al., 2001), obtained when a subject is lying at rest during a scan. This network consists of low-frequency oscillations consisting of interconnected regions that include the medial prefrontal cortex, parts of the parietal lobe and the posterior cingulate cortex (Buckner et al., 2008). Study of the default mode network is considered important because it is hypothesized to have interactions with the sensory, motor, and emotional systems. In addition, alterations to this system has been implicated in different diseases and disorders (Buckner et al., 2008). While the default mode network has been found in humans, it has also been described in nonhuman primates (NHPs) (Rilling et al., 2007, Hutchison et al., 2011) and rats (Lu et al., 2012).

Various imaging studies in NHPs have been conducted to identify the default mode network. As shown in Table 1, these studies have been conducted in various species, but mainly the rhesus macaque. Resting state functional connectivity studies in the rhesus macaque have identified various regions that are highly correlated with the BOLD signal in the precuneus. Across these studies, the posterior cingulate cortex, medial prefrontal cortex, regions of the parietal cortex and the temporal regions are areas identified as the default mode network (Vincent et al., 2007, Margulies et al., 2009, Mantini et al., 2011). Moreover, functional connectivity studies in chimpanzees demonstrated high activity in the medial prefrontal cortex and the medial parietal cortex (Rilling et al., 2007). Independent component analysis in the anesthetized rhesus macaque identified several subnetworks associate with the default mode network. These regions included the arcuate sulcus, posterior cingulate, precuneus, areas in the visual cortex, superior temporal gyrus, insular cortex, anterior cingulate, orbitofrontal cortex, postcentral and precentral gyrus (Hutchison et al., 2011). These findings are important because many of the identified regions appear in the default mode network in humans.

Animal models are an indispensable resource in basic research and are useful for determining the effects of pharmacological treatment on performance of complex behavioral tasks and cognition (Granholt, 2010). In particular, NHPs bridge the gap between rodent and human clinical studies and provide a unique translational resource. The use of NHPs allows complete control over experimental conditions (e.g., nutritional status, housing conditions, medical history, etc.), which is often difficult to accomplish in clinical studies. Although some neuroimaging studies have sought to identify the default mode network in NHPs, these studies have typically used fMRI or functional connectivity with seed analysis (Vincent et al., 2007, Margulies et al., 2009, Mantini et al., 2011). Such analyses only provide a localized view of brain organization; an alternative approach is to use network science to determine the functional organization across the whole brain.

Network science is an emerging field that envisions the brain as a complex system with global integration and regional specialization (Watts and Strogatz, 1998). Network science utilizes the concepts of graph theory, which conceives of the brain as a set of nodes (or vertices) connected by edges. Nodes in a network represent voxels or brain regions, while edges represent a physical or functional connection between nodes. This model is appealing because the brain is often viewed as a system containing multiple regions with specialized functions that rapidly interact with each other to produce complex behaviors. Early applications of network science in the brain were done on histological samples in the rhesus macaque cortex (Jouve et al., 1998, Sporns et al., 2007, Sporns et al., 2002) while the first human studies were conducted using structural MRI scans (Rivière et al., 2002). These early studies focused on structural topology, but later studies integrated fMRI to better understand the functional organization of the human brain (Eguíluz et al., 2005). As the field has progressed, many tools and concepts have been developed for neuroimaging in humans (Bullmore and Sporns, 2009, Telesford et al., 2011, Rubinov and Sporns, 2010). However,

functional brain networks have not been applied to the NHP brain. Human studies can often introduce confounding factors that can be controlled in NHP studies, making development of an animal brain model appealing for use in brain network studies. The focus of this paper is to evaluate NHP brain networks to understand the basic functional organization of the primate brain and to show how changes in pharmacological conditions can affect the brain network.

Methods

Animals

Baseline resting state data was acquired in an adult, male rhesus macaque across two scans within the same session to determine network variability. Briefly, the animal was sedated with ketamine and transported to the scanner where he inhaled 3% isoflurane via a nose cone until intubated. Once intubated, he was maintained under 1.5% isoflurane for the remainder of the scan session. The animal was artificially ventilated in order to control the physiological parameters. Expired CO₂ levels and respiration rate were monitored using a Capnomac monitor (Datex-Engstrom Division Instrumentarium Corp., Helsinki, Finland). Heart rate and oxygen saturation levels were monitored using a pulseoximeter. The animal's body temperature was maintained using warmed blankets. Resting state fMRI data was then acquired as described below. The identical procedures were utilized to acquire resting state fMRI data in 10 adolescent, male vervet monkeys. Finally, in order to validate our findings, the same rhesus monkey underwent a separate challenge study to determine the impact of acute ethanol intoxication on brain networks. Following acquisition of baseline resting state data, the animal was challenged with an acute intravenous infusion of 1.0 g/kg ethanol (equivalent of 4 drinks) administered over an 8 minute period. Additional series of fMRI data were acquired post-infusion. All animals were recovered from anesthesia, extubated and provided pipeline oxygen until recovered and returned to their home cage.

The vervet monkeys in this study were sourced from an ongoing study to determine the neurobiological consequences of chronic alcohol self-administration. These animals were scanned in the naïve state prior to ethanol exposure. The rhesus macaque had a previous history of drug exposure. This animal was utilized in a pilot study to explore the feasibility of applying network science to demonstrate the effects of a pharmacological challenge on brain networks.

Scanning protocol

Functional magnetic resonance imaging (fMRI) data was collected in two NHP species: one (n=1) rhesus macaque (*Macaca mulatta*) and ten (n=10) vervet monkeys (*Chlorocebus aethiops*). Scans were acquired on a 3.0T GE Scanner equipped with a Twin Speed Gradient Coil in Zoom Mode (maximum gradient strength 4G/cm and a slew rate of 150mT/m/ms). Resting state fMRI data was acquired with a gradient echo EPI sequence using an 8-channel receive only RF head coil with a form factor designed specifically for the nonhuman primate (Dr. Cecil Hayes, University of Washington). Imaging data was acquired as axial oblique contiguous slices oriented along a line that extends from the superior surface of the anterior commissure to the center of the posterior commissure (AC/PC line) with frequency direction Right to Left. The rhesus macaque was scanned over two separate sessions; the first was a within session scan comparison of hub structure (gradient echo EPI, 64×64 acquisition matrix, acquisition resolution=2.19mm×2.19mm×3.0mm, FOV=14cm×14cm, flip angle=58°, TE=28ms, TR=1150ms, 400 volumes); the second session was an acute ethanol challenge with the following parameters adapted from (Murnane and Howell, 2010): gradient echo EPI, 64×48 acquisition matrix, acquisition resolution=1.88mm×1.88mm×4.0mm, FOV=14cm×14cm, flip angle=70°, TE=40ms,

TR=1000ms, 512 volumes. The first scan for the rhesus macaque was adapted from a human protocol and further optimized for the second scan. The vervet monkeys were scanned in the treatment-naïve condition as part of an ongoing study of the neurobiological consequences of long-term ethanol self-administration. Parameters were based on an optimized sequence developed for NHPs (gradient echo EPI, 96×48 acquisition matrix, acquisition resolution=1.5mm×1.5mm×1.5mm, FOV=19.2cm×19.2cm, flip angle=90°, TE=20ms, TR=4000ms, 124 volumes). An anatomic scan (T1-weighted 3D-SPGR, 256×256 acquisition matrix, 0.5mm slice thickness, TE=3.432ms, TI=600ms, TR=8.16ms) was collected for normalization of functional images. All scans were conducted at Wake Forest School of Medicine, Winston-Salem, NC. All procedures were approved by the Institutional Animal Care and Use Committee and the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23).

Data analysis

Functional images were motion corrected, normalized to the NITRC (Neuroimaging Informatics Tools and Resources Clearinghouse) macaque and vervet template for the respective animal species. Tissue maps from the NITRC template were used to mask imaging data for gray matter, white matter, and cerebrospinal fluid (CSF). For the rhesus macaque, white matter and CSF maps were thresholded at 0.8 and used to calculate the mean signal from each tissue type. White matter maps were thresholded at 0.8 and CSF maps were thresholded at 0.7 for the vervet monkeys. The gray matter maps were thresholded at 0.2 and used to mask the functional images. The threshold for gray matter is more lenient than those used for white matter and CSF to ensure the inclusion of the majority of gray matter voxels. Functional and anatomic scans were skull-stripped using 3D Slicer (<http://www.slicer.org>) (Gering et al., 1999, Pieper et al., 2004, Pieper et al., 2006), and anatomic scans were normalized to the NITRC template using FSL with normalization parameters subsequently applied to the functional scans.

Network analysis

As diagrammed in Figure 1, fMRI time courses were extracted for each voxel and corrected for physiological noise by band-pass filtering to remove signal from the range of 0.009-0.08 Hz (Fox et al., 2005, van den Heuvel et al., 2008). Mean time courses from the entire brain (the average of voxel values within the brain parenchyma mask including gray and white matter), the deep white matter, and the ventricles were regressed from the filtered time series. The six rigid-body motion parameters from the motion correction process were also regressed from the time series.

A correlation matrix was produced by computing the Pearson correlation between all possible pairs of voxels within the fMRI time series. A threshold was applied to the correlation matrix, whereby voxel pairs above the threshold were considered functionally connected and assigned a value of 1 and voxel pairs below the threshold were considered not connected and assigned a value of 0. The discretization of the correlation matrix produces a binary adjacency matrix. The threshold was defined such that the relationship between the number of nodes and average number of connections between nodes was consistent across subjects. Specifically, the relationship $S = \log(N)/\log(K)$ was the same across subjects, where N is the number of nodes in the entire network, K is the average degree of the network, and S represents the average path length of an Erdős-Rényi network (Newman et al., 2001). For this paper, we chose the threshold $S = 2.5$ to our define networks, but network properties have been demonstrated to be robust for different S values (Hayasaka and Laurienti, 2010).

Graph metric analysis

From the adjacency matrix, the following graph metrics were calculated at each node and averaged to yield *average graph metrics* for the entire network: degree (K), clustering coefficient (C), minimum path length (L), local efficiency (E_{loc}), and global efficiency (E_{glob}). Network metrics used in this study are shown in Table 1 (for more details on specific metrics, see (Rubinov and Sporns, 2010)). Degree for each voxel was mapped to anatomic space to determine the most connected regions, or hubs, within the brain. Hub maps were generated with degree comprising the top 20% of the most connected nodes. These maps provide the anatomic location of hubs and demonstrate the functional organization, or hub structure, of the brain.

Community structure analysis

In addition to average graph metrics, community structure analysis detects the level of interconnectedness in the network. Nodes that are more interconnected with each other than with the rest of the network form communities or modules. These modules segregate the network into small compartments that not only highlight the most connected nodes in the network, but also highlight the functional organization of the network across the whole brain (Fortunato, 2010, Girvan and Newman, 2002, Meunier et al., 2009). Community structure was determined using *Qcut*, an algorithm that uses spectral graph partitioning and local optimization to determine the community structure of the brain (for more details on this method, see (Ruan and Zhang, 2008)). Community structure was used in this study to determine how modular structure was affected by acute ethanol exposure. Community structure is assessed using Q , which measures the quality of the partition (Newman, 2006). A higher value of Q produces more accurate partitioning of the network, while a lower value of Q indicates a network that resembles a random network.

Results

Within animal graph metric analysis and hub structure in the rhesus macaque

An analysis was conducted within the same rhesus monkey across separate fMRI runs to determine within session variability. Average graph metrics showed no significant differences between runs for clustering coefficient, path length, local efficiency and global efficiency (Figure 2a). Plots of the degree distribution show that both runs followed a truncated power law and appeared similar between runs (Figure 2b). Hub overlap maps of the rhesus macaque found prominent hubs in the medial prefrontal cortex, cingulate cortex, temporal lobes and visual cortex (Figure 3a). Scans within the same session show that hubs appeared in the same regions on both runs (Figure 3b). Combining both runs, an overlap image was produced showing a high level of consistency between the first run (in red) and second run (in blue) with overlaps across runs (in purple) (Figure 3c). These results suggest that hub structure is a stable feature of brain organization. To ensure that consistency of hubs is not an artifact of the threshold, binary images showing the consistency of runs were produced at different thresholds of high degree nodes (Figure 4a). Across thresholds, the percentage of voxels that overlap for a single run range from 54% at the most liberal threshold to 41% at the most stringent threshold (Figure 4b).

Across thresholds, it appears that nodes in the temporal lobes were consistent at the most stringent thresholds. As the threshold became more stringent this high level of overlap remained, suggesting that network hubs are stable across scans. In addition, community structure analysis revealed that these hubs formed modules consistent with the default mode network as described in the literature. A comparison of the Q -values for Run 1 ($Q = 0.60$) and Run 2 ($Q = 0.63$) further emphasized the stability of the community structure across scans. Across both runs, two stable modules were identified: the first included the medial

prefrontal cortex and the second encompassed the precuneus, cingulate and paracentral lobule (Figure 5).

Network comparison across an animal population

Based on the consistent results within the same animal across runs, we then conducted an analysis of the same metrics across a group of animals. The high level of agreement seen within subject was also seen across animals as observed in the cohort of 10 treatment-naïve vervets. Top overlap maps showed hubs in the medial prefrontal cortex, anterior cingulate, cingulate cortex, visual cortex, parietal lobe and temporal lobe, particularly the superior temporal gyrus (Figure 6). These hubs were consistent with hubs seen in the rhesus macaque, which suggests consistent brain organization across species. An analysis of average graph metrics also showed that across the population, no animal significantly deviated from the group and the degree distribution was consistent across the population (Figure 7).

Changes in the brain network following acute ethanol intoxication

The above studies were conducted to establish feasibility of conducting network analysis in the NHP brain under baseline conditions. A subsequent study was conducted to evaluate the utility of resting state brain network analyses for studies of acute pharmacological challenges. Data were collected during the resting state and again following an acute ethanol infusion. Average graph metrics pre- and post- ethanol infusion were not significantly different across runs (Figure 8); however, a comparison of hub maps showed a dramatic shift in the location of hubs in the brain (Figure 9a). At baseline, hubs appeared to encompass the default mode network with prominent hubs in the parietal lobe, visual cortex, medial frontal cortex and temporal lobes. After acute administration of ethanol, these hubs largely disappeared and new hubs were scattered through the brain. An analysis of the community structure supports this change in hub structure; a module containing the areas consistent with the default mode network at baseline showed connections across a host of brain regions post-ethanol (Figure 9b). Comparing the Q -values pre- and post-ethanol, there was a decrease following acute ethanol from 0.58 to 0.49. This change indicates that the network exhibits more properties of a random network after the ethanol infusion,

Discussion

Hub structure in the NHP brain

Although there has been extensive investigation of networks in humans, to our knowledge, there have been no studies investigating functional brain networks in NHPs using network science. While traditional imaging studies focus on regional activation or independent subnetworks, network science offers the ability to determine how different regions of the brain interact. Nonetheless, previous studies evaluating default-mode organization in NHPs still provide valuable information about brain organization. As seen in the Table 2, studies using different species and experimental conditions have identified various regions as part of the default mode network. However, despite these differences, several regions appear across animals, notably the precuneus, cingulate cortex, medial prefrontal cortex, and superior temporal gyrus. In our study, hubs in the brain networks from rhesus macaque and vervet monkeys were found in the medial prefrontal cortex, cingulate cortex, visual cortex and temporal lobes. Hubs in the visual cortex appear to be more pronounced in the vervet animals, while hubs in the cingulate cortex of the rhesus macaque were more pronounced. These findings may reflect species differences, but may also be a result of sample size as the rhesus macaque reflects a single animal. Despite these differences, the hubs found here are consistent with findings in previous NHP imaging studies.

While the default mode network in NHPs is similar to that in humans, there are some regions that only consistently appear in the NHP brain network. Hubs in the visual cortex and the temporal lobes, particularly the superior temporal gyrus, have been noted in NHP networks in this study and previous studies, but not in humans (Hutchison et al., 2011, Margulies et al., 2009, Vincent et al., 2007). Nonetheless, hubs in the precuneus, cingulate cortex, and medial prefrontal cortex complement those found in humans, which lends credence for the use of such animal models for clinical application.

Another major finding in this study is that under anesthesia, community structure organization suggests that the medial prefrontal cortex and posterior cingulate do not exist within the same community, which lends support to previous studies citing a lack of connectivity between the two regions (Raichle et al., 2001, Vincent et al., 2007, Mantini et al., 2011). In this study, parietal-prefrontal connectivity formed a single community during one scan in the rhesus macaque; however, scans in the vervet population and subsequent scans in the rhesus macaque failed to reproduce this community. While these results do not rule out a connection between the medial prefrontal cortex and posterior cingulate, they suggest that under anesthesia, connections between these regions may be impacted. It is worth noting that other studies have identified links between these regions in the default mode (Hutchison et al., 2011), thus further investigation of the effects of anesthesia on the NHP brain is warranted.

These results, particularly the decoupling of the parietal and frontal regions may be a result of anesthesia. Studies in humans have reported that the prefrontal cortex is sensitive to anesthesia, namely, local connectivity is reduced under light anesthesia conditions (Deshpande et al., 2010). Nonetheless, despite a potential uncoupling of long range connections between the parietal and prefrontal cortex, the regions associated with the default mode network still appear as major hubs in the network. These results suggest that a NHP model, even under conditions of anesthesia, can be useful for understanding neurological disorders that affect humans.

Graph metric reproducibility

A chief concern is the reliability of graph metrics across scans. In this study, reproducibility of hubs was found to be high across studies and across subjects. These results reflect findings observed in human fMRI networks (Telesford et al., 2010); however, it should be noted that high reproducibility in the human study was confined to hubs in the network. Many studies in brain networks focus on location and changes to hub structure of networks (Bullmore and Sporns, 2009, Buckner et al., 2009, Achard et al., 2006).

It should also be noted that the sample size in this study, which is representative for studies that utilize NHPs, is considerably smaller than typical human studies. Nonetheless, given the strong agreement comparing graph metrics, degree distribution, hub localization and community structure, it appears that reproducibility in this study are consistent with results found in human. The preservation of hub structure sets a baseline for a population and how a particular state or condition can change in this network organization. These results suggest that focusing on changes in hub structure and connectivity can provide a greater understanding of how a particular substance or state can affect the organization of the brain network.

Measuring changes in brain organization due to alcohol

One powerful aspect of this network model is the ability to see changes in brain organization due to changes in conditions. Functional connectivity studies have also shown that acute and chronic alcohol use can disrupt functional subnetworks in the human brain (Chanraud et al.,

2011, Rzepecki-Smith et al., 2010). However, to our knowledge, there are no studies that show global effects of alcohol across the whole brain. Additionally, to our knowledge, this is the first study to report changes in brain networks in the NHP brain following an ethanol challenge. In this study, we see the disruption of the resting state network across the entire brain. The disruption in the network is best exemplified by a lower value of Q for community structure. The post-ethanol network displayed properties more associated with a random network. In terms of the brain, a network with more random properties lacks the regional specialization considered critical in small-world networks. These results highlight some of the benefits of network analysis since changes in whole brain connectivity can be explored without prior knowledge of the region that is altered by a given challenge. Although the current study tracked changes in brain networks following an acute ethanol challenge, these data set the foundation for future studies to investigate network connectivity following chronic ethanol exposures.

Limitations

One important caveat of this study is that the macaque used in these investigations had a drug history from previous studies, thus there is a chance that the pharmacological effects of previous treatments may have impacted the brain networks. This limitation also makes it difficult to describe the effects of alcohol on the default mode network, as the drug history of the animal may play a factor in the observed changes. Despite this limitation, this investigation is still useful as the main goal of this study was to demonstrate the ability to build these networks and show that this method is sensitive to changes induced by the introduction of ethanol. This result is best demonstrated by the within animal reproducibility that shows stable hub structure across scans and altered hub structure during the ethanol scan. In addition, the vervet population provided a treatment-naïve data set and there continued to be a high overlap of network structure between species. These findings suggest that hubs found in both species are representative of the default mode network in NHPs.

While average graph metrics across experimental groups can show significant differences between networks or groups of networks, there may be a limitation to comparing graph metrics alone. A lack of change across scans does not necessarily indicate that the compared networks are the same. For instance, studies have shown no significant differences between graph metrics, but have shown dramatic changes in network topology (Burdette et al., 2010, Moussa et al., 2011). In such cases, it is essential to conduct further analyses that include localization of hubs and analysis of network community structure.

Another aspect of brain network studies is that they may not provide great specificity with regard to discrete brain regions. Many BOLD fMRI studies highlight specific brain regions or cortical areas based on changes in activity. In contrast, network science places less emphasis on specific brain regions and focuses on global interactions across the brain. It is worth noting that the power of network science lies in its ability to address the complexity in the brain as a whole, thus traditional t-tests may not be ideal for such analyses (Telesford et al., 2011).

Another concern with graph metric analysis lies in methodology, particularly related to network parcellation. For network parcellation, most studies use voxel-based or regions of interest (ROI) networks. In voxel-based networks, each voxel represents a node (Eguíluz et al., 2005, van den Heuvel et al., 2008), while ROI networks segment the brain based on an atlas, such as the Automated Anatomical Labeling (AAL) (Tzourio-Mazoyer et al., 2002). A previous study comparing different network parcellation methods showed that ROI networks can underrepresent high degree nodes (Hayasaka and Laurienti, 2010). Moreover, they showed that the removal of local connections in voxel-based networks does not significantly change the degree distribution of the network, suggesting that hubs found in voxel-based

networks are true local connections. Another issue that arises is the classification of voxels based on tissue type. In our network analysis, tissue probability maps for gray matter, white matter and CSF were used to identify nodes. At coarser resolutions, nodes can be misclassified and included in the analysis. Consequently, this can result in the appearance of hubs in regions outside of gray matter, often at the interface of gray and white matter. While there is still no consensus on which method is most appropriate, we advocate the use of voxel-based networks with total brain coverage as it allows for investigation of interregional connectivity and avoids the removal of signal that may be critical to accurate network representation.

Conclusion

Network science offers a great opportunity to understand the brain as a complex system and continues to gain popularity in the neuroimaging community. With its increasing popularity, there will also be a greater effort to apply network science to animal models. One of the main benefits of an animal model is the ability to tightly control conditions that are often confounding factors in a human study. In our investigation, we have successfully applied network science techniques to a NHP model in multiple species and have found results that were not only consistent across two NHP species, but were also consistent with previous animal studies as well as with human studies. Following the baseline scan sessions, the animals were subjects in a chronic ethanol self-administration paradigm using a well-established model of self-administration (Vivian et al., 2001). This model has been reported to elicit heavy drinking in cynomolgus and rhesus macaques paralleling the patterns and levels of intake observed in humans. Further, this design was recently shown to have predictive value in determining which animals would ultimately become heavy drinkers at the end of the self-administration study (Grant et al., 2008). The ability to correlate changes in functional connectivity following long-term exposure will likely provide valuable insight into how chronic alcohol impacts brain networks and subsequent cognitive processes.

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Figure 1. Schematic of network and reproducibility analysis

Voxel time courses are extracted from the fMRI time series and computing the Pearson correlation between all voxel pairs, a correlation matrix is produced. A threshold is applied to the correlation matrix to generate a binary adjacency matrix. From the adjacency matrix, graph metrics are calculated at every node in the network and averaged to produce mean graph metrics.

Figure 2. Graph metric reproducibility in rhesus macaque

Average graph metrics in rhesus macaque show no significant differences across fMRI runs for clustering coefficient (C , $p=0.92$), path length (L , $p=0.70$), local efficiency (E_{loc} , $p=0.94$), global efficiency (E_{glob} , $p=0.39$). A plot of the degree distribution shows both runs are similar and follow a truncated power law.

Figure 3. Hub maps of top 20% of nodes of highest degree in rhesus macaque

(a-b) Hubs appear in the medial prefrontal cortex, cingulate cortex, temporal lobe and visual cortex. (c) Comparing the two runs, nodes from Run 1 (red) were overlaid onto nodes in Run 2 (blue). Regions appearing in purple highlight the overlap of high degree nodes across both runs.

Figure 4. Binary hub maps for two fMRI runs across thresholds

(a) Binary images of the network hubs were generated for each run with an increasing threshold from the top 25% to 10% of the most connected nodes. Nodes only in the first run appear in blue, nodes only in the second run appear in green, while nodes in both runs appear in red. (b) The percentage overlap shows that as much as 54% down to 41% of the nodes for either run is shared. Across both maps, high degree hubs appear to be stable across run with no major differences at increasing threshold levels.

Figure 5. Module organization of default mode communities in rhesus macaque

Two modules were found to be associated with the default mode network. However, in this study, regions in the premedial frontal cortex were not connected to the precuneus/posterior cingulate cortex.

Figure 6. Overlap map across 10 vervet monkeys

Hub maps of top 20% high degree nodes were overlaid to determine areas with a high consistency of high degree nodes. Hubs were found in the medial prefrontal cortex, anterior cingulate, cingulate cortex, visual cortex, parietal lobe, temporal lobe, and superior temporal gyrus.

Figure 7. Average graph metrics across vervet monkey cohort

Across subjects, average metrics were found to be highly reproducible across animals. The degree distribution for each animal also appear to be similar and follow a truncated power law.

Figure 8. Average graph metrics pre- and post- ethanol bolus

Average metrics did not significantly differ across metrics for clustering coefficient (C , $p=0.70$), path length (L , $p=0.71$), local efficiency (E_{loc} , $p=0.83$), global efficiency (E_{glob} , $p=0.70$). The degree distribution does not appear to significantly change across runs.

Figure 9. Network changes in rhesus macaque brain following ethanol challenge

A rhesus macaque was given 1 g/kg of ethanol after an initial scan. (a) As seen in the hub map, the network is altered with much of the hub structure disappearing (b) While the community organization shows regions associated with the default mode network before the ethanol bolus, no discernible pattern.

Table 1

Default mode network studies in NHP. Description of regions identified as default mode network in NHPs in previous publications and this study.

Publication	NHP Type	Default Mode Network Regions
Rilling, 2007	Chimpanzee	medial prefrontal cortex medial parietal cortex
Vincent, 2007	Rhesus macaque	precuneus posterior cingulate cortex dorsal medial prefrontal cortex lateral temporoparietal cortex superior temporal gyrus posterior parahippocampal cortex
Marguiles, 2009	Rhesus macaque	precuneus superior parietal cortex paracentral lobule motor cortex dorsolateral prefrontal cortex dorsomedial prefrontal cortex multimodal lateral inferior parietal cortex visual cortex
Hutchison, 2011	Rhesus macaque	arcuate sulcus posterior cingulate precuneus cortex areas in the visual cortex superior temporal gyrus insular cortex anterior cingulate orbitofrontal cortex postcentral gyrus precentral gyrus
Mantini, 2011	Rhesus macaque	posterior cingulate cortex medial prefrontal cortex dorsolateral prefrontal cortex insula/operculum lateral parietal cortex
Current study	Rhesus macaque	medial prefrontal cortex cingulate cortex temporal lobes visual cortex

Publication	NHP Type	Default Mode Network Regions
Current study	Vervet	medial prefrontal cortex
		anterior cingulate
		cingulate cortex
		visual cortex
		parietal lobe
		superior temporal gyrus

Table 2

Description and mathematical definition of graph metrics used in this study.

Graph metric	Description	Equation
Degree	Measures the connectivity of each node	$k_i = \sum_{j=1}^n A_{ij}$
Clustering coefficient	Measures local neighborhood connectivity, calculated as the likelihood that neighbors of a node are also neighbors	$C_i = \frac{2e_i}{k_i(k_i - 1)}$
Path length	Measures the distance between nodes in the network and is calculated as the shortest geodesic distance between each node and every other node	$L = \frac{1}{N(N-1)} \sum_{ij \in n, i \neq j} d_{ij}$
Global efficiency	Measures the closeness of an individual node to all other nodes in the network	$E_{glob} = E(G) = \frac{1}{N(N-1)} \sum_{i \neq j \in G} \frac{1}{d_{ij}}$
Local efficiency	Describes how interconnected the neighbors are in a particular area of the network	$E_{loc} = \frac{1}{N} \sum_{i \in n} E(G_i)$