Published in final edited form as:

Alcohol. 2019 February ; 74: 95–104. doi:10.1016/j.alcohol.2018.04.007.

Low Cognitive Flexibility as a Risk for Heavy Alcohol Drinking in Non-human Primates

Tatiana A Shnitko¹, Steven W. Gonzales¹, and Kathleen A. Grant^{1,2}

¹Division of Neuroscience, Oregon National Primate Research Center, Oregon Health & Science University, 505 NW 185th Avenue, Beaverton, OR 97006-3448, USA

²Department of Behavioral Neuroscience, Oregon Health & Science University, 3181 SW Sam Jackson Park Road, L-470, Portland, OR, 97239-3098, USA.

Abstract

Chronic alcohol abuse is frequently considered a habitual or inflexible behavior; however, measures of preexisting cognitive flexibility prior to initiation of alcohol use are usually not available. This study used rhesus monkeys and a set-shifting task to investigate if preexisting cognitive flexibility would predict increased risk for heavy alcohol drinking. As previously reported, monkeys were given 30 daily set-shifting sessions prior to alcohol access. These sessions consisted of the same sequence of 8 unique visual discriminations (sets) of two objects that varied on two dimensions (shapes and colors). The ratio of errors per trials, session duration, and maximum set reached were primary dependent variables from each session and were used to comprise a session performance index (PI) that ranged from a low performance PI of 31 to an optimal performance PI of 247. Here animals underwent an alcohol induction period followed by 22 weeks of daily (22-h) self-administration sessions with free access to water and alcohol. Based on average daily alcohol intake during 22 weeks of 22-h/day access, the monkeys were categorized as non-heavy (mean=2.0±0.3 g/kg/day; n=3) and heavy (mean=3.3±0.5 g/kg/day; n=6) drinkers. The two groups diverged in performance on the set-shifting task across the 30 pre-alcohol sessions, and at the end of the pre-alcohol testing, the group average PI was 216±27 and 137±71 for the future non-heavy and heavy drinkers, respectively. The data show that low cognitive flexibility assessed with a set-shifting procedure was predictive of future classification as a heavy alcohol drinker. The data highlight individual differences in both cognitive flexibility and in alcohol self-administration in this population of rhesus monkeys.

Keywords

set-shifting abilities; alcohol abuse; non-human primates; risk factors; cognitive flexibility

Corresponding author: Kathleen A. Grant PhD, ONPRC, grantka@ohsu.edu, Phone: (503) 346-5461, Fax: (503) 346-5513.

Conflict of interests

Authors have no conflict of interests to disclose.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Behavioral flexibility is described as an ability to adapt to changing sets of rules governing learned associations between conditioned stimuli and optimal responses. Cognitive flexibility relies on high order brain functions such as sensory perception, response inhibition, and associative learning and can be used to index inflexible or habitual behaviors (Brown & Tait, 2010). Excessive use of alcohol and other substances is frequently considered a habitual behavior that is resistant to modification (Barker & Taylor, 2014; Corbit & Janak, 2016; Corbit et al., 2012; Fanelli et al., 2013; McKim et al., 2016). Animal studies show that chronic alcohol and drug abuse can impair measures of cognitive flexibility. For instance, rodent and nonhuman primate (NHP) studies have shown that deficits in cognitive control and flexibility are highly associated with chronic use of nicotine (Ortega et al., 2013), methamphetamine (Cox et al., 2016), cocaine (Kromrey et al., 2015; Porter et al., 2011) and alcohol (Barker et al., 2017; Fernandez et al., 2017; Trantham-Davidson et al., 2014). Similar results from human subject studies showed impaired set-shifting and reversal abilities in nicotine (Nesic et al., 2011), cocaine (Verdejo-Garcia et al., 2015) and alcohol users (Trick et al., 2014; Wolff et al., 2016). However, data linking preexisting measures of cognitive flexibility, as measured by set shifting, to the risk for development of excessive alcohol drinking associated with alcohol dependence are lacking.

There are several ways to measure cognitive flexibility in humans and animals. Predominantly, variations of the Wisconsin Card Sorting Test (WCST) that uses intradimensional-extradimensional shifts in serial visual discrimination sets are applied across species (Brown & Tait, 2016; Floresco et al., 2008; Roberts et al., 1988; Shnitko et al., 2017). Specifically, an “attentional set” of stimuli is presented where the relevant dimension of each stimulus (for example, the shape or the color) is learned by a trial and error approach. Once the performance reaches preset criteria for acquisition (e.g., a ratio of correct to incorrect trials), the relevant dimension can be changed, which then requires a subject’s “attentional shift” to respond correctly (e.g., an alternative shape becomes the correct stimulus; an example of an intradimensional shift), or another dimension of the stimulus can become the basis of the discrimination (e.g., color rather than shape becomes the correct stimulus dimension; an example of an extradimensional shift). In general, intradimensional reversals or extradimensional shifts produce more errors than new intradimensional sets (e.g. Crofts et al., 1999; Weed et al., 2008).

Attentional set-shifting procedures to measure cognitive flexibility have been applied to NHPs using visual stimuli and touch-screens. In a variation of the procedures described above, the touch screen can be integrated into a side-wall of the NHP housing cage. Further, simultaneous assessment of individual set-shifting ability within a group of monkeys is possible (Shnitko et al., 2017). Thus the acquisition and progression through multiple sets of visual discriminations can be directly compared within and across subjects in this group setting. Measures of cognitive flexibility in NHPs correspond to WCST metrics for assessment of cognitive flexibility in human subjects. For example, the number of categories completed in human studies are equivalent to discrimination sets in NHP studies, and the number of trials administered and number of errors or correct responses are equivalent in both human and NHP tests (for example, Kohli & Kaur, 2006). Those metrics can be

combined into a compound “WCST factor score” to estimate a relationship between cognitive flexibility (the factor score) and a status of a subject (e.g., functional status of a patient after stroke (Greve et al., 1999) or drinking status as in present study). Thus the compound metric of cognitive flexibility can be compared among a cohort of monkeys with the same history of opportunities to learn and perform, which can be viewed much like a classroom setting.

As demonstrated by longitudinal and cross-sectional studies, academic performance in secondary school (age 13-18) is predictive of alcohol drinking status (Bergen et al., 2005; Heradstveit et al., 2017). However, because these studies rely on self-reported alcohol intakes and academic grades and do not account for school dropouts, the actual risk is hard to evaluate (Balsa et al., 2011). The present study addressed if set-shifting performance can predict subsequent chronic heavy drinking in a NHP model of voluntary alcohol self-administration. Macaque monkeys provide a unique animal model for studying risk of heavy alcohol drinking because of their similarity to humans in genetic, endocrinological, neuroanatomical and metabolic systems (Grant & Bennett, 2003; Grant, Stafford, et al., 2008; Jimenez & Grant, 2017). Further, macaques show wide individual differences in the propensity to daily drinking to intoxication using a well-established protocol of alcohol self-administration (Allen et al., 2018; Baker et al., 2014; Baker et al., 2017; Grant, Leng, et al., 2008). Because both the self-administration of alcohol and the set-shifting ability of these monkeys show individual differences, we explored the hypothesis that poor cognitive performance on the set-shifting task prior to alcohol access would predict increased risk for future classification as a heavy drinker.

Material and methods

Animals

Late adolescent / young adult male rhesus monkeys (cohort 14, *Macaca mulatta*, n=9) obtained from the breeding colony of Oregon National Primate Research Center were enrolled in the study at 4 to 5 years of age and 5 to 6 kg in body weight. The monkeys were relocated to an indoor housing room with controlled temperature (20-22°C), humidity (65%), and an 11-h light cycle with lights on at 07:00 a.m. Each monkey was housed in a metal cage (0.8 × 0.8 × 0.9 m) capable of vertical partition removal to allow housing in pairs. Monkeys were paired for 1-2 hour/day. The monkeys were fed a diet of nutritionally complete 1 g banana-flavored pellets (TestDiet, USA) and fresh fruit. Food and fluid availability was dependent on experimental phase and described below. All monkeys were weighed weekly and ethanol intakes based on body weight (g/kg) were calculated from the contemporary weights. All procedures were conducted according to the Guide for the Care and Use of Laboratory Animals (National Research Council (US) Committee, 2011) and approved by the Oregon National Primate Research Center Animal Care and Use Committee.

Equipment

Each housing cage was equipped with an operant panel as previously described (Shnitko et al., 2017; Vivian et al., 2001). Each panel included a centrally located dowel and 2 drinking

spouts, and below one of the spouts there was a food receptacle connected to a 1 g pellet dispenser (Med Associates Inc., USA) and below the receptacle there was a recessed well that could detect the insertion of a finger via the breaking of an infrared beam. Each spout was connected via tubing to a plastic bottle placed on a digital scale (Ohaus Adventurer, USA) located outside the cage. The bottle contained either filtered tap water or 4% ethanol (w/v diluted in water), refilled with fresh solutions daily. Additionally, a computer-controlled LCD monitor (11×13.25 inch, Dell Inc., Model E1715S) with attached touch-sensitive screen overlay (Keytec, Inc., Model OPTIR Touch PPMT, USA) was incorporated to each panel. All programming used a National Instruments interface and LabView software (LabView 2011, SP1, National Instruments, TX, USA). Thus, the operant panels were used for the cognitive testing, ethanol and water self-administration and food delivery. To initiate food or fluid delivery, the dowel had to be pulled (closing an electrical circuit) and held (see Grant, Leng, et al., 2008 for details). To operate the touch screen no dowel response was required.

Set-shifting procedure

The set-shift procedure has been described in detail (Shnitko et al., 2017). After the initial learning phase of 5-7 sessions, the performance of each monkey on the set-shifting task was acquired over 30 consecutive sessions prior to any ethanol exposure (Figure 1A). Briefly, the set-shifting sessions were self-pacing with a maximum time of 45-min, and began daily at 10am. Every session started with a presentation of a photograph of a nature scene (e.g., trees and sky). Touching the photograph was required to initiate the first and all subsequent trials. Each trial consisted of the display of two geometrical shapes and color combinations and a trial ended after a monkey touched one of the shapes or 30 sec elapsed. Animals had to discriminate which shape was predetermined as correct or incorrect stimulus. Touching the correct stimulus within 30 seconds resulted in the 2-sec presentation of a photograph pre-screened to be preferred (fruit, lab personnel, or affiliative; for details see Shnitko et al., 2017). Touching the incorrect stimulus resulted in a blank screen for 10 sec followed by a new trial of the same shapes and color combinations. Locations of the correct and incorrect shapes on the screen (left or right) were randomly altered. Every third correct trial ended with the delivery of a 1 g banana-flavored food pellet. Correct responses (trials) did not have to be consecutive to be reinforced. The stimulus discrimination was defined as acquired once 12 correct trials occurred within a running total of 15 trials. Once a discrimination was acquired, the next discrimination set was presented on the next trial. The number of trials to acquire a discrimination was not limited within the confines of the 45-min session.

Daily sessions consisted of the same sequence of discrimination sets. The session began with a simple discrimination between two shapes; both shapes were either solid black or white. When the original discrimination was acquired then the reversal of that discrimination was initiated in which the opposite shape was assigned as correct. This set and reversal was followed by the presentation of two compound stimuli (two new shapes and two new colors) with shape as the correct dimension and then the reversal of this discrimination. This set and reversal was then followed by two new compound stimuli, again with the correct dimension being the shape of the object (also known as an intradimensional shift in the discrimination) and the reversal of the correct shape. Finally, there was another set of two new unique object

shapes and colors, however this discrimination was based on the color of the object rather than the shape (an extradimensional shift in the discrimination) and reversal of the correct color if the original discrimination was acquired. The session would end upon successful completion of the final set reversal or 45 min after the session onset.

Alcohol self-administration

Induction of alcohol self-administration—Alcohol self-administration begun after set-shift testing was completed. A schedule-induced polydipsia procedure was used to induce monkeys to drink 4% (w/v) ethanol as previously described (Grant, Leng, et al., 2008; Vivian et al., 2001). Briefly, daily induction sessions were given in which 1 gram banana flavored pellets were delivered under a fixed-time of 300 sec until a required volume of either water or 4% ethanol was consumed. Initially, monkeys were induced to drink water for 30 sessions, and then water was replaced with 4% ethanol. Monkeys were then required to consume the ethanol solution at the volume sufficient to obtain 0.5 g/kg, 1.0 g/kg and 1.5 g/kg of ethanol. When animal consumed the required volume, the ethanol spout became inoperative but water was available through the other spout, and the remaining daily ration of food became available two hours later (Grant et al., 2008).

Open-access alcohol self-administration—After the induction period, the daily sessions consisted of concurrent access to water and 4% (w/v) alcohol for 22 h/day. Within this session, the daily food ration was divided equally into three “meals” available under a fixed ratio 1 schedule. The first meal was available at the beginning of session and subsequent meals were available at 2 h intervals (additional details in Baker et al., 2014; Grant, Leng, et al., 2008). Drinking and eating measurements (volume consumed from each spout and each food pellet delivery) were marked in session time for subsequent data analysis. Animals were allowed 22 weeks of concurrent access to water and 4% (w/v) ethanol for 22 h/day, 7 days a week, total of 154 sessions.

Blood ethanol concentration (BEC) analysis—Monkeys were trained to present their leg through an opening in the front wall of the housing cage and comply with veinipuncture without the use of anesthesia. The blood samples were collected from the saphenous vein 30, 60, 90 min after start of the induction sessions and 7 h after start of the 22 h drinking sessions in 4-5 day intervals. The blood samples were collected throughout the 3 months of ethanol induction period and 22 weeks of daily 22 h access to ethanol. The ethanol content was assessed by gas chromatography (5890 Series II, Hewlett-Packard, Avondale, PA).

Data analysis—Daily ethanol intakes (g/kg) during approximately 22 weeks of 22 h/day ethanol access and BECs were used to categorize individual monkeys as low, binge, heavy and very heavy drinkers (Baker et al., 2014). Low and binge drinkers were then combined into a “non-heavy” drinking group whereas the monkeys categorized as heavy and very heavy drinkers were combined into a “heavy” drinking group as previously described (Cervera-Juanes et al., 2016; Grant, Leng, et al., 2008; Helms, Park, et al., 2014a; Helms, Rau, et al., 2014b). Dependent variables of alcohol self-administration were ethanol intake (ml/day) as a proportion of total fluid intake (ethanol and water ml/day), maximum ethanol bout in ml (bout was defined as ethanol intake without a 5-min pause in drinking) and

latency to the maximal ethanol bout in sec (a session maximum bout was defined as the largest volume (ml) consumed without a 5-min pause in drinking in the 22 hr session).

One of the important aspects of a set-shift procedure used in this study is that within a single session, monkeys progressed through the discriminations from a simple to an extradimensional reversal set at their own pace and abilities. There were apparent individual differences in the ability to progress through the task. For example, comparing two monkeys with a similar number of errors recorded during the same session did not reflect what maximal sets a monkey achieved during that session (Shnitko et al., 2017). Thus individual abilities to progress through discrimination sets were explored as a predictor of drinking status in this study. For each session and monkey, cognitive flexibility was assessed with a performance index (PI) as previously described (Shnitko et al., 2017). Briefly, three variables that were recorded during each session (number of errors/trials, session duration and maximal set reached) were scaled from 0 to 100 and summed to derive a PI. A linear mixed model analysis was used to estimate whether set-shift performance of monkeys measured prior to any ethanol exposure varied in the groups of heavy and non-heavy drinkers (SPSS Statistics, 24, IBM Corp.) In this analysis, the individual PIs from each session were averaged across 5-session blocks for each subject and used as the dependent variable. Group (heavy vs non-heavy drinker) and session-block were used as fixed factors, and monkey was used as a random factor. Besides the PI, the number of perseverative errors was analyzed in order to estimate a level of perseveration that occurred during performance on the set-shift task. The perseverative error was defined as consecutive incorrect trials following more than 3 incorrect consecutive trials (for example: correct – error – error – error – error – error – correct; total number of errors=5, number of perseverative errors= 2). Thus continuous perseveration (repetition of incorrect response without interruption) was assessed (Sandson & Albert, 1984).

Interaction between set-shifting abilities and alcohol self-administration indices was estimated using Spearman's rank correlation. The correlation coefficients were estimated between an individual performance index averaged across the last ten set-shifting sessions prior to alcohol induction and the drinking parameters across the approximate 22 weeks of daily drinking sessions for each individual monkey. Median of the latency to maximal ethanol bout, median of the size of maximal ethanol bout and average daily ethanol intakes were used in this analysis. Significance of the correlations was confirmed with two-tailed t-test. Data were considered significant with $p < 0.05$ for all analysis.

Results

Estimation of drinking categories for individual subjects.

The range of average daily ethanol intake during 22-h open access was 1.64-4.02 g/kg/day (Figure 1B). Based on the individual ethanol intakes, the monkeys were categorized as low/binge ($n=3$), and heavy/very heavy ($n=6$) drinkers as defined in Baker et al., 2014. Briefly, the heavy/very heavy demarcation of drinking uses the percentage of heavy drinking sessions (>3.0 g/kg for $>20\%$ of sessions, Figure 1C; Baker et al., 2014). The average 22-h ethanol intake in the heavy drinkers was 3.29 ± 0.2 g/kg/day and resulted in BEC of 102 ± 10 mg% at 7 h after the session onset. Average 22-h ethanol intake among non-heavy drinkers

was 2.03 ± 0.2 g/kg/day with associated average BECs of 29 ± 4 mg% at 7 h after the session onset.

Analysis of cognitive flexibility as a predictor of drinking status in monkeys.

To compare set-shifting ability across monkeys, a PI, based on the ratio of errors to trials, set reached and time to finish the daily session, was calculated for each monkey and session across 30 test-sessions (Figure 2). As shown in Figure 2A, there was no systematic difference between future non-heavy and heavy drinking groups prior to ethanol self-administration as indicated by averaged PI 96 ± 33 and 80 ± 16 , respectively. The groups diverged in their performance over the subsequent sessions and the group average PI for the 30th session was 216 ± 27 and 137 ± 71 for the future non-heavy and heavy drinkers, respectively. For statistical analyses, individual PI for each monkey were averaged across 5 session blocks (Figure 2B). A linear mixed model analysis revealed a significant main effect of block ($F_{5,7}=71.7$, $p<0.0001$) and group by block interaction ($F_{5,7}=13.2$, $p<0.01$) and no main effect of group ($F_{1,7}=1.8$, $p=0.22$). As Figure 2B shows, both groups progressed through the training and significantly improved their performance by the fourth block of testing compared to block 1 (baseline) (Bonferroni t-test, $p<0.05$). However, future heavy drinkers then regressed and did not perform above baseline in blocks 5 and 6 (Bonferroni t-test, $p>0.05$), whereas the future non-heavy drinkers maintained the improved performance from baseline over the last 3 session-blocks (Bonferroni t-test, $p<0.05$).

An analysis of each orthogonal dependent variable of the performance index (the ratio of trials ending in error to the total number of trials, the session duration and the maximal set the animal reached during the session) is given in Figures 2C-H. Over the 30 sessions, the average error ratio and the duration of sessions decreased, while the average set reached during the sessions increased (Figure 2C, E and G). The average error ratio per session was similar between the two groups of monkeys at block 1 (0.46 for future non-heavy and 0.47 for future heavy drinkers) and decreased by the end of testing (block 6: 0.33 and 0.4 for future non-heavy and future heavy drinkers, respectively, Figure 2D). A mixed model analysis yielded significant main effect of block ($F_{5,7}=7.3$, $p<0.05$) and no main effect of group ($F_{1,7}=2.2$, $p=0.2$) or group by block interaction and ($F_{5,7}=1.5$, $p=0.3$). Similar results were observed for session duration. Monkeys from both groups used all allowed session time (2700 s) at the beginning of testing (block 1) and completed the sessions on average of 1835 ± 330 s (future non-heavy drinkers) and 2345 ± 194 s (future heavy drinkers) at the end of testing (block 6, Figure 2F). A mixed model analysis revealed significant main effect of block ($F_{5,35}=9.1$, $p<0.0001$) and no effect of group ($F_{1,7}=3.3$, $p=0.1$) or group by block interaction ($F_{5,35}=1.3$, $p=0.22$). In terms of the set reached by either group over the blocks of sessions (Figure 2H), future non-heavy drinkers were able to consistently reach the final set (set 8: the reversal for extradimensional shift) during the last half of sessions (blocks 4-6), while the group of future heavy drinkers on average only reached the extradimensional shift (set 7). Overall, while each of the factors used here to measure performance on the set-shifting is informative of cognitive flexibilities, only a combination of these factors into a single performance index differentiated future drinking status.

Analysis of cognitive flexibility as a predictor of drinking patterns in monkeys.

In addition to the daily ethanol intake (g/kg/day) during 22-h drinking sessions, non-heavy and heavy drinkers differ in their patterns of drinking, consistent with previous reports (Baker et al., 2014; Grant, Leng, et al., 2008). Specifically, heavy drinkers have higher frequencies and larger volumes of the ethanol bouts, a consumption pattern that results in higher BECs compared to non-heavy drinkers (Baker et al., 2017). A typical 22-h session is represented for one of the heavy drinkers in this study in Figure 3A. As demonstrated in the graph, the animal consumed 691.8 ml of 4% ethanol solution (27.5% of total fluid intake) in the session, and 36.6% (253.4 ml) of the total ethanol was consumed during the lights off period (from 18:00 to 7:00, 7-20 h in the session). The animal ingested both water and ethanol in large bouts early in the session (Figure 3B) and consumed his total ethanol volume in 20 bouts across the 22 h (Figure 3C). The average ethanol bout size for this animal during this single session was 34.6 ml, however the maximal bout was 189.7 ml.

To evaluate if performance on the set-shift task predicts the patterns of ethanol consumption, performance indexes were averaged across the 10 last sessions for each monkey. A linear regression analysis was used to estimate the relationship between the PI and session ethanol preference (ethanol intake/total fluid during session), latency to the maximal ethanol bout (sec) and size of the maximal ethanol bout (ml) (Figure 3D-F). There was a strong negative correlation between PI and ethanol preference (Spearman's rank correlation, $r=-0.72$, $p<0.05$). Correlation between PI and volume of maximal ethanol bout appeared to be negative; however, it did not reach significance as estimated by two-tailed t-test (Spearman's rank correlation, $r=-0.66$, $p=0.05$). Thus, higher ethanol preference is associated with lower performance index. On the other hand, higher performance index was associated with longer delays to the maximal ethanol bout within the session (Spearman's rank correlation, $r=0.70$, $p<0.05$). Table 1 demonstrates parameters of ethanol self-administration recorded for each individual subject (Supplemental materials).

Analysis of perseverative errors as a predictor of drinking status in monkeys.

The number of perseverative errors that occurred during each of the sets were added across each session and averaged within groups of future non-heavy and heavy drinkers (Figure 4A). Similar to the errors to trials ratio, the number of perseverative errors significantly declined from session 1 to session 30 in both groups of monkeys (effect of session $F_{29,203}=4.1$, $p<0.01$; effect of group $F_{1,7}=0.01$, $p=0.9$ and group $\times$ session $F_{29,203}=0.9$, $p=0.6$). The percentage of perseverative errors from the total number of errors was consistent across the sessions and groups (Figure 4B). Finally, the average portion of perseverative errors was $19.7\pm2.5\%$ and $19.4\pm3.3\%$ in the group of future heavy and non-heavy drinkers, respectively. Importantly, number of perseverative errors strongly correlated with performance index in 8 out of 9 subjects (Spearman's rank order correlation analysis revealed $r<-0.5$ and $p<0.01$).

Finally, the number of errors that occurred only during the reversal sets were compared between the future heavy and non-heavy drinkers (Supplemental Figure 1). In the group of future heavy drinkers, the number of perseverative errors on reversal sets ranged from 28 ± 10 during simple discrimination reversal set to 17 ± 5 during reversal to extradimensional shift.

In the group of future non-heavy drinkers, the number of perseverative errors ranged from 17 ± 5 to 13 ± 6 during the same sets. A mixed model analysis revealed no significant main effect of group ($F_{1,3}=1$, $p=0.3$) and no significant effect of set ($F_{3,2}=3.4$, $p=0.2$) or group by set interaction ($F_{3,2}=3.4$, $p=0.7$).

Discussion

The current study of rhesus monkeys in an alcohol self-administration procedure shows that cognitive flexibility assessed with a set-shifting procedure was lower in a group of future heavy alcohol drinkers compared to a group of future non-heavy alcohol drinkers (Figure 2A). This finding highlights individual differences in both cognitive flexibility and in alcohol self-administration in this population of rhesus monkeys (Baker et al., 2014; Shnitko et al., 2017). The monkeys in this study were given cognitive testing and then induced to drink alcohol at age of 4.5-5.5 years, which is an age that is considered late adolescence in non-human primates (Barr et al., 2004; Colman & Anderson, 2011). In agreement with our previous research, the onset of alcohol drinking at this late adolescent age resulted in a high proportion 67% (6 out of 9) of the subjects meeting the criteria for heavy or very heavy drinkers (Figure 2, Baker et al., 2017). In terms of pre-alcohol cognitive flexibility, the future categorical drinking groups diverged in the rate at which they increased their performance over daily set-shifting sessions.

Because low performance on set-shifting is believed to reflect an aspect of habitual behavior that is more under the control of the prevailing stimuli than the outcome of the behavior (Corbit & Janak, 2016), these data suggest that a predisposition towards habitual behaviors is a risk for future heavy drinking. Further, the aspects of alcohol self-administration that were correlated with the pre-alcohol performance (PI) reflect the differentiation of “gulpers” from “sippers” with higher volumes of ethanol in drinking bouts and shorter latency to the daily maximal bout (Figure 4, Grant, Leng, et al., 2008). The consumption pattern of high volumes and short latencies is also associated with higher BECs (Grant et al., 2008) and with ethanol-induced changes in striatal output that favors sensorimotor circuitry over associative circuitry in the control of behavior (Cuzon Carlson et al., 2017; Cuzon Carlson et al., 2011). Thus, it appears that predisposition to repetitive, efficient sensorimotor-based actions, as evidenced in both a cognitive task and in response to alcohol, capture a neural basis for risk towards chronic excessive alcohol drinking.

The measure of performance index (PI) was derived from the combination of three orthogonal measures, and it is important to note that any single measure of cognitive flexibility in this set-shifting task did not differentiate the future drinking status. Traditionally, WCST assess perseveration that occurs while a subject performs the task. Perseveration characterizes a behavior that is repeated unintentionally in the presence of a negative feedback and is regarded as a reflection of frontal dysfunction. However, deficits in performance on WCST are also dependent on occurrence of non-perseverative errors as they preclude a subject from reaching a preset criteria for progression through discrimination sets (for example, *randome errors* Barcelo & Knight, 2002). In this study we found no evidence that perseverative errors (a traditional WCST metric) would predict future drinking status of NHP (Figure 4A), even though individual PI correlated with perseveration in 8 out of 9

subjects (Figure 4C). One of 3 variables that is included in the PI is derived from number of errors recorded during each session. We found that consistently throughout 30 sessions, about 19% of errors were perseverative (Figure 4B), and therefore, strong correlations between the PI and the number of perseverative errors is not surprising as errors to trial ratio declined throughout 30 sessions as the PI increased (Figure 2C). Analysis of errors recorded at reversal sets is a common measure of perseveration during the set-shift performance in animal studies. However, this metric did not reveal any difference between the future heavy and non-heavy drinkers (Supplemental Figure 1). Thus, analysis of perseverative errors is useful in assessing cognitive flexibility (Izquierdo et al., 2017); however, the present data suggest that this metric failed as a predictor of chronic alcohol drinking under this set-shifting procedure in monkeys. Future studies will explore consequences of chronic alcohol self-administration on perseveration.

Overall, cognitive ability accompanied by brain maturation during adolescence is associated with excessive alcohol intake. For instance, human studies show that decreased academic and cognitive performance by secondary school students, as measured by grade point average and school attendance, is associated with alcohol use (Balsa et al., 2011; Heradstveit et al., 2017). Recent cross-sectional and longitudinal studies revealed that heavy alcohol use during adolescence amplifies decline in gray matter volume of frontal and temporal cortical areas and attenuates white matter development in the pons and the corpus callosum (Pfefferbaum et al., 2017; Squeglia et al., 2015). However, there are very few longitudinal studies that address the role of pre-existing executive functions (e.g., attention, inhibition, working memory) as potential risk factors for further heavy alcohol consumption. One study by Goudriaan and coauthors (2011) demonstrated that male students with poor performance on a decision-making task measured during their second year of college predicted heavy alcohol use during a follow-up two years later (Goudriaan et al., 2011). In contrast, response inhibition did not predict an alcohol use in these subjects. Squeglia and coauthors (2015) studied alcohol-naïve early adolescent subjects using battery of tests to assess cognitive functioning on six cognitive domains (inhibition, short-term memory, attention, verbal learning and memory, visuospatial functioning and planning, and problem solving abilities) and found that only poor inhibition control was a predictor of higher lifetime alcohol use and higher peak number of drinks per occasion by ages 17 to 18 (Squeglia et al., 2014). On the other hand, Boelema and coauthors (2016) reported that poor executive function (inhibition, working memory, shift of visual attention, and sustained visual attention) at the age of 11 years does not predict alcohol use in 16-year old subjects (Boelema et al., 2016). One of the downsides in these studies is that they rely on self-reported alcohol use from teenage populations. Together with other aspects of assessing the relationship of alcohol use and cognitive abilities in late adolescence and young adulthood, the data can lead to conflicting conclusions (Balsa et al., 2011). Here, the self-administration model was predicated on precise measurements of alcohol intake patterns and blood alcohol concentrations along with control over other variables such as nutrition and absence of other psychoactive substances.

There are a few laboratory animal studies that have addressed *pre-existing* cognitive deficits as predictors of alcohol abuse, and those that have, focused on impulsivity associated with genetic predisposition for heavy alcohol consumption in rodents (Beckwith & Czachowski, 2014, 2016; Oberlin & Grahame, 2009; Wilhelm & Mitchell, 2008). The present study

provides the first evidence that a comparatively low level of cognitive flexibility in a cohort of non-human primates is associated with developing heavy alcohol intake (Figure 2). Importantly, all monkeys were assessed under a “classroom” setting, where there was a consistent history of training and testing sessions that allows for direct comparison of individual differences in acquisition and performance on the cognitive task. Additionally, the consistent history of alcohol induction and then nearly continuous access to alcohol also reveals individual differences in daily consumption. Together, the data provide a unique opportunity to explore the association of cognitive flexibility with future alcohol drinking patterns.

The translational significance to human studies emphasize sensorimotor processes in adolescents as a window to a predisposition towards habitual drinking patterns of large volumes of alcohol in single bouts that reliably lead to high BEC levels and alcohol intoxication (Cuzon Carlson et al., 2011). It is very well documented that adolescence is a critical period for the cortical development, as maturation within the prefrontal and orbitofrontal cortices including myelination, synaptic elimination and overall white-matter growth occurs intensively during this period (for review, Toga et al., 2006). Lesion and neuroimaging studies demonstrated that cognitive flexibility requires intact control from prefrontal cortices (for review Alvarez & Emory, 2006). Thus, the low performance on the cognitive task of future heavy drinkers observed in the present study may be due to incomplete cortical maturation. Future studies designed to link pre-existing cognitive flexibility and measures of cortical maturation will help define mechanisms of brain circuitry related to risk for heavy drinking.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding

This study was funded by the National Institute on Alcohol Abuse and Alcoholism (U01 AA013510 (to KAG), P60 AA010760 (to KAG) and R24 AA019431 (to KAG).

References

- Guide for the Care and Use of Laboratory Animals, 8th (2011) (8th ed.). Washington (DC).
- Allen DC, Gonzales SW, & Grant KA (2018). Effect of repeated abstinence on chronic ethanol self-administration in the rhesus monkey. *Psychopharmacology (Berl)*, 235(1), 109–120. doi:10.1007/s00213-017-4748-9 [PubMed: 29051997]
- Alvarez JA, & Emory E (2006). Executive function and the frontal lobes: a meta-analytic review. *Neuropsychol Rev*, 16(1), 17–42. doi:10.1007/s11065-006-9002-x [PubMed: 16794878]
- Baker EJ, Farro J, Gonzales S, Helms C, & Grant KA (2014). Chronic alcohol self-administration in monkeys shows long-term quantity/frequency categorical stability. *Alcohol Clin Exp Res*, 38(11), 2835–2843. doi:10.1111/acer.12547 [PubMed: 25421519]
- Baker EJ, Walter NA, Salo A, Rivas Perea P, Moore S, Gonzales S, & Grant KA (2017). Identifying Future Drinkers: Behavioral Analysis of Monkeys Initiating Drinking to Intoxication is Predictive of Future Drinking Classification. *Alcohol Clin Exp Res*, 41(3), 626–636. doi:10.1111/acer.13327 [PubMed: 28055132]

- Balsa AI, Giuliano LM, & French MT (2011). The effects of alcohol use on academic achievement in high school. *Econ Educ Rev*, 30(1), 1–15. doi:10.1016/j.econedurev.2010.06.015 [PubMed: 21278841]
- Barcelo F, & Knight RT (2002). Both random and perseverative errors underlie WCST deficits in prefrontal patients. *Neuropsychologia*, 40(3), 349–356. [PubMed: 11684168]
- Barker JM, Bryant KG, Osborne JI, & Chandler LJ (2017). Age and Sex Interact to Mediate the Effects of Intermittent, High-Dose Ethanol Exposure on Behavioral Flexibility. *Front Pharmacol*, 8, 450. doi:10.3389/fphar.2017.00450 [PubMed: 28736526]
- Barker JM, & Taylor JR (2014). Habitual alcohol seeking: modeling the transition from casual drinking to addiction. *Neurosci Biobehav Rev*, 47, 281–294. doi:10.1016/j.neubiorev.2014.08.012 [PubMed: 25193245]
- Barr CS, Schwandt ML, Newman TK, & Higley JD (2004). The use of adolescent nonhuman primates to model human alcohol intake: neurobiological, genetic, and psychological variables. *Ann N Y Acad Sci*, 1021, 221–233. doi:10.1196/annals.1308.027 [PubMed: 15251892]
- Beckwith SW, & Czachowski CL (2014). Increased delay discounting tracks with a high ethanol-seeking phenotype and subsequent ethanol seeking but not consumption. *Alcohol Clin Exp Res*, 38(10), 2607–2614. doi:10.1111/acer.12523 [PubMed: 25335779]
- Beckwith SW, & Czachowski CL (2016). Alcohol-Preferring P Rats Exhibit Elevated Motor Impulsivity Concomitant with Operant Responding and Self-Administration of Alcohol. *Alcohol Clin Exp Res*, 40(5), 1100–1110. doi:10.1111/acer.13044 [PubMed: 27028842]
- Bergen HA, Martin G, Roeger L, & Allison S (2005). Perceived academic performance and alcohol, tobacco and marijuana use: longitudinal relationships in young community adolescents. *Addict Behav*, 30(8), 1563–1573. doi:10.1016/j.addbeh.2005.02.012 [PubMed: 16122617]
- Boelema SR, Harakeh Z, van Zandvoort MJ, Reijneveld SA, Verhulst FC, Ormel J, & Vollebergh WA (2016). Executive functioning before and after onset of alcohol use disorder in adolescence. A TRAILS study. *J Psychiatr Res*, 78, 78–85. doi:10.1016/j.jpsychires.2016.03.014 [PubMed: 27086184]
- Brown VJ, & Tait DS (2010). Behavioral Flexibility: Attentional Shifting, Rule Switching and Response Reversal.
- Brown VJ, & Tait DS (2016). Attentional Set-Shifting Across Species. *Curr Top Behav Neurosci*, 28, 363–395. doi:10.1007/7854_2015_5002 [PubMed: 26873018]
- Cervera-Juanes R, Wilhem LJ, Park B, Lee R, Locke J, Helms C, ... Ferguson B. (2016). MAOA expression predicts vulnerability for alcohol use. *Mol Psychiatry*, 21(4), 472–479. doi:10.1038/mp.2015.93 [PubMed: 26148813]
- Colman RJ, & Anderson RM (2011). Nonhuman primate calorie restriction. *Antioxid Redox Signal*, 14(2), 229–239. doi:10.1089/ars.2010.3224 [PubMed: 20698791]
- Corbit LH, & Janak PH (2016). Habitual Alcohol Seeking: Neural Bases and Possible Relations to Alcohol Use Disorders. *Alcohol Clin Exp Res*, 40(7), 1380–1389. doi:10.1111/acer.13094 [PubMed: 27223341]
- Corbit LH, Nie H, & Janak PH (2012). Habitual alcohol seeking: time course and the contribution of subregions of the dorsal striatum. *Biol Psychiatry*, 72(5), 389–395. doi:10.1016/j.biopsych.2012.02.024 [PubMed: 22440617]
- Cox BM, Cope ZA, Parsegian A, Floresco SB, Aston-Jones G, & See RE (2016). Chronic methamphetamine self-administration alters cognitive flexibility in male rats. *Psychopharmacology (Berl)*, 233(12), 2319–2327. doi:10.1007/s00213-016-4283-0 [PubMed: 27037939]
- Crofts HS, Muggleton NG, Bowditch AP, Pearce PC, Nutt DJ, & Scott EA (1999). Home cage presentation of complex discrimination tasks to marmosets and rhesus monkeys. *Lab Anim*, 33(3), 207–214. doi:10.1258/002367799780578174 [PubMed: 10780838]
- Cuzon Carlson VC, Grant KA, & Lovinger DM (2017). Synaptic adaptations to chronic ethanol intake in male rhesus monkey dorsal striatum depend on age of drinking onset. *Neuropharmacology*. doi:10.1016/j.neuropharm.2017.12.010
- Cuzon Carlson VC, Seabold GK, Helms CM, Garg N, Odagiri M, Rau AR, ... Grant KA. (2011). Synaptic and morphological neuroadaptations in the putamen associated with long-term, relapsing

- alcohol drinking in primates. *Neuropsychopharmacology*, 36(12), 2513–2528. doi:10.1038/npp.2011.140 [PubMed: 21796110]
- Fanelli RR, Klein JT, Reese RM, & Robinson DL (2013). Dorsomedial and dorsolateral striatum exhibit distinct phasic neuronal activity during alcohol self-administration in rats. *Eur J Neurosci*, 38(4), 2637–2648. doi:10.1111/ejn.12271 [PubMed: 23763702]
- Fernandez GM, Lew BJ, Vedder LC, & Savage LM (2017). Chronic intermittent ethanol exposure leads to alterations in brain-derived neurotrophic factor within the frontal cortex and impaired behavioral flexibility in both adolescent and adult rats. *Neuroscience*, 348, 324–334. doi:10.1016/j.neuroscience.2017.02.045 [PubMed: 28257889]
- Floresco SB, Block AE, & Tse MT (2008). Inactivation of the medial prefrontal cortex of the rat impairs strategy set-shifting, but not reversal learning, using a novel, automated procedure. *Behav Brain Res*, 190(1), 85–96. doi:10.1016/j.bbr.2008.02.008 [PubMed: 18359099]
- Goudriaan AE, Grekin ER, & Sher KJ (2011). Decision making and response inhibition as predictors of heavy alcohol use: a prospective study. *Alcohol Clin Exp Res*, 35(6), 1050–1057. doi:10.1111/j.1530-0277.2011.01437.x [PubMed: 21332527]
- Grant KA, & Bennett AJ (2003). Advances in nonhuman primate alcohol abuse and alcoholism research. *Pharmacol Ther*, 100(3), 235–255. [PubMed: 14652112]
- Grant KA, Leng X, Green HL, Szeliga KT, Rogers LS, & Gonzales SW (2008). Drinking typography established by scheduled induction predicts chronic heavy drinking in a monkey model of ethanol self-administration. *Alcohol Clin Exp Res*, 32(10), 1824–1838. doi:10.1111/j.1530-0277.2008.00765.x [PubMed: 18702645]
- Grant KA, Stafford J, Thiede A, Kiley C, Odagiri M, & Ferguson B (2008). Who is at risk? Population characterization of alcohol self-administration in nonhuman primates helps identify pathways to dependence. *Alcohol Res Health*, 31(4), 289–297. [PubMed: 23584006]
- Greve KW, Bianchini KJ, Hartley SM, & Adams D (1999). The Wisconsin Card Sorting Test in stroke rehabilitation: factor structure and relationship to outcome. *Arch Clin Neuropsychol*, 14(6), 497–509. [PubMed: 14590577]
- Helms CM, Park B, & Grant KA (2014). Adrenal steroid hormones and ethanol self-administration in male rhesus macaques. *Psychopharmacology (Berl)*, 231(17), 3425–3436. doi:10.1007/s00213-014-3590-6 [PubMed: 24781519]
- Helms CM, Rau A, Shaw J, Stull C, Gonzales SW, & Grant KA (2014). The effects of age at the onset of drinking to intoxication and chronic ethanol self-administration in male rhesus macaques. *Psychopharmacology (Berl)*, 231(8), 1853–1861. doi:10.1007/s00213-013-3417-x [PubMed: 24448900]
- Heradstveit O, Skogen JC, Hetland J, & Hysing M. (2017). Alcohol and Illicit Drug Use Are Important Factors for School-Related Problems among Adolescents. *Front Psychol*, 8, 1023. doi:10.3389/fpsyg.2017.01023 [PubMed: 28676779]
- Izquierdo A, Brigman JL, Radke AK, Rudebeck PH, & Holmes A (2017). The neural basis of reversal learning: An updated perspective. *Neuroscience*, 345, 12–26. doi:10.1016/j.neuroscience.2016.03.021 [PubMed: 26979052]
- Jimenez VA, & Grant KA (2017). Studies using macaque monkeys to address excessive alcohol drinking and stress interactions. *Neuropharmacology*, 122, 127–135. doi:10.1016/j.neuropharm.2017.03.027 [PubMed: 28347838]
- Kohli A, & Kaur M (2006). Wisconsin Card Sorting Test: Normative data and experience. *Indian J Psychiatry*, 48(3), 181–184. doi:10.4103/0019-5545.31582 [PubMed: 20844649]
- Kromrey SA, Gould RW, Nader MA, & Czoty PW (2015). Effects of prior cocaine self-administration on cognitive performance in female cynomolgus monkeys. *Psychopharmacology (Berl)*, 232(11), 2007–2016. doi:10.1007/s00213-015-3865-6 [PubMed: 25633093]
- McKim TH, Bauer DJ, & Boettiger CA (2016). Addiction History Associates with the Propensity to Form Habits. *J Cogn Neurosci*, 28(7), 1024–1038. doi:10.1162/jocn_a_00953 [PubMed: 26967944]
- Nesic J, Rusted J, Duka T, & Jackson A (2011). Degree of dependence influences the effect of smoking on cognitive flexibility. *Pharmacol Biochem Behav*, 98(3), 376–384. doi:10.1016/j.pbb.2011.01.015 [PubMed: 21281663]

- Oberlin BG, & Grahame NJ (2009). High-alcohol preferring mice are more impulsive than low-alcohol preferring mice as measured in the delay discounting task. *Alcohol Clin Exp Res*, 33(7), 1294–1303. doi:10.1111/j.1530-0277.2009.00955.x [PubMed: 19389183]
- Ortega LA, Tracy BA, Gould TJ, & Parikh V (2013). Effects of chronic low- and high-dose nicotine on cognitive flexibility in C57BL/6J mice. *Behav Brain Res*, 238, 134–145. doi:10.1016/j.bbr.2012.10.032 [PubMed: 23103711]
- Pfefferbaum A, Kwon D, Brumback T, Thompson WK, Cummins K, Tapert SF, ... Sullivan EV (2017). Altered Brain Developmental Trajectories in Adolescents After Initiating Drinking. *Am J Psychiatry*, appiajp201717040469. doi:10.1176/appi.ajp.2017.17040469
- Porter JN, Olsen AS, Gurnsey K, Dugan BP, Jedema HP, & Bradberry CW (2011). Chronic cocaine self-administration in rhesus monkeys: impact on associative learning, cognitive control, and working memory. *J Neurosci*, 31(13), 4926–4934. doi:10.1523/JNEUROSCI.5426-10.2011 [PubMed: 21451031]
- Roberts AC, Robbins TW, & Everitt BJ (1988). The effects of intradimensional and extradimensional shifts on visual discrimination learning in humans and non-human primates. *Q J Exp Psychol B*, 40(4), 321–341. [PubMed: 3145534]
- Sandson J, & Albert ML (1984). Varieties of perseveration. *Neuropsychologia*, 22(6), 715–732. [PubMed: 6084826]
- Shnitko TA, Allen DC, Gonzales SW, Walter NA, & Grant KA (2017). Ranking Cognitive Flexibility in a Group Setting of Rhesus Monkeys with a Set-Shifting Procedure. *Front Behav Neurosci*, 11, 55. doi:10.3389/fnbeh.2017.00055 [PubMed: 28386222]
- Squeglia LM, Jacobus J, Nguyen-Louie TT, & Tapert SF (2014). Inhibition during early adolescence predicts alcohol and marijuana use by late adolescence. *Neuropsychology*, 28(5), 782–790. doi:10.1037/neu0000083 [PubMed: 24749728]
- Squeglia LM, Tapert SF, Sullivan EV, Jacobus J, Meloy MJ, Rohlfing T, & Pfefferbaum A (2015). Brain development in heavy-drinking adolescents. *Am J Psychiatry*, 172(6), 531–542. doi:10.1176/appi.ajp.2015.14101249 [PubMed: 25982660]
- Toga AW, Thompson PM, & Sowell ER (2006). Mapping brain maturation. *Trends Neurosci*, 29(3), 148–159. doi:10.1016/j.tins.2006.01.007 [PubMed: 16472876]
- Trantham-Davidson H, Burnett EJ, Gass JT, Lopez MF, Mulholland PJ, Centanni SW, ... Chandler LJ (2014). Chronic alcohol disrupts dopamine receptor activity and the cognitive function of the medial prefrontal cortex. *J Neurosci*, 34(10), 3706–3718. doi:10.1523/JNEUROSCI.0623-13.2014 [PubMed: 24599469]
- Trick L, Kempton MJ, Williams SC, & Duka T (2014). Impaired fear recognition and attentional set-shifting is associated with brain structural changes in alcoholic patients. *Addict Biol*, 19(6), 1041–1054. doi:10.1111/adb.12175 [PubMed: 25123156]
- Verdejo-Garcia A, Clark L, Verdejo-Roman J, Albein-Urios N, Martinez-Gonzalez JM, Gutierrez B, & Soriano-Mas C. (2015). Neural substrates of cognitive flexibility in cocaine and gambling addictions. *Br J Psychiatry*, 207(2), 158–164. doi:10.1192/bjp.bp.114.152223 [PubMed: 26045346]
- Vivian JA, Green HL, Young JE, Majerksy LS, Thomas BW, Shively CA, ... Grant KA (2001). Induction and maintenance of ethanol self-administration in cynomolgus monkeys (*Macaca fascicularis*): long-term characterization of sex and individual differences. *Alcohol Clin Exp Res*, 25(8), 1087–1097. [PubMed: 11505038]
- Weed MR, Bryant R, & Perry S (2008). Cognitive development in macaques: attentional set-shifting in juvenile and adult rhesus monkeys. *Neuroscience*, 157(1), 22–28. doi:10.1016/j.neuroscience.2008.08.047 [PubMed: 18805462]
- Wilhelm CJ, & Mitchell SH (2008). Rats bred for high alcohol drinking are more sensitive to delayed and probabilistic outcomes. *Genes Brain Behav*, 7(7), 705–713. doi:10.1111/j.1601-183X.2008.00406.x [PubMed: 18518928]
- Wolff N, Gussek P, Stock AK, & Beste C (2016). Effects of high-dose ethanol intoxication and hangover on cognitive flexibility. *Addict Biol* doi:10.1111/adb.12470

Highlights

- A novel set-shifting procedure was used to explore poor cognitive flexibility as a risk for development of excessive alcohol drinking.
- A non-human primate model of alcohol self-administration was used.
- Cognitive flexibility assessed with the set-shifting procedure was lower in a group of future heavy alcohol drinkers.
- Preexisting low cognitive flexibility predicts increased risk for classification as a heavy drinker in non-human primates.

Figure 1.

Ethanol consumption in heavy and non-heavy drinkers. A. Experimental timeline. Dashed boxes represent periods of set-shift testing in monkeys. Solid boxes represent periods of water (blue) and ethanol (red) self-administration. Triangle shapes indicate size of the discriminative stimuli presented to monkeys during set-shift sessions. B. Ethanol consumption in heavy and non-heavy drinkers measured during 22-h drinking sessions. Median of ethanol intake analyzed across 154 sessions within 22 weeks period is shown for each individual monkey. C. Percentage of 22-h drinking session when monkeys consumed ethanol at dose over 3 g/kg. Four drinking categories were estimated based on daily ethanol intake and depicted in color (for details see Baker et al., 2014).

Figure 2.

Performance on the set-shifting task prior to alcohol drinking in the groups of future heavy and non-heavy alcohol drinkers. A. Performance index was calculated for each individual monkey for every session based on 3 factors: number of errors to trials ratio, session duration and set reached during the session (for details see Shnitko et al., 2017). Bold red and blue lines indicate data as group mean of PIs estimated for each subject within a group. Dashed red and blue lines indicate $\pm$ SD estimated for both groups. B. Individual PI were averaged within 5 sessions (block) for each monkey and then averaged within each group (data are mean $\pm$ SEM). Changes in error to trial ratio, duration and set reached across 30 sessions or 6 blocks of sessions are shown in graphs C-H. Data for error to trial ratio and duration in C and E are presented as mean (bold lines) $\pm$ SD (dashed lines) and as mean $\pm$ SE in D and F. Median set reached during a session and within a block are shown in G and H. The * indicates blocks when averaged performance index was significantly different ($p < 0.05$) from 1st block within the future non-heavy and future heavy drinkers, respectively as estimated by post-hoc analysis with Bonferroni t-test.

Figure 3.

Correlation between performance on the set-shifting task and attributes of alcohol self-administration. (A-C) Representation of a single 22-hr alcohol drinking session and 1 hour into the drinking obtained in a heavy drinking monkey. The water and ethanol intakes are depicted in blue and red along with the percentage of water and 4% (w/v) ethanol solution from the total fluid intake in A. Examples of water and ethanol bouts are demonstrated in B, and number and size of the bouts occurred during the session are shown in C, where red bar represent the maximal ethanol bout across the session. (D-F) The attributes of alcohol

drinking: the percentage of 4% (w/v) ethanol solution from the total fluid intake, latency to the bout, maximal ethanol bout and were obtained for each individual monkey across all drinking sessions. Spearman's rank correlation analysis and two-tailed t-test were used to evaluate relationship between the variables.

Figure 4.

Perseverative errors recorded during the set-shift task. A. Number of perseverative errors across 30 sessions averaged within groups of future heavy and non-heavy drinkers. B. Percent of perseverative errors from total number of errors per session collapsed across subjects in each group. * $p < 0.05$ (linear mixed model analysis). C. Correlation between performance index and perseveration assayed in individual animals. Spearman's rank correlation coefficient was estimated for 30 test sessions for each individual animal and p confirmed with two-tailed t -test ($r < -0.54$, $p < 0.01$, $n = 8$ monkeys out of 9). Colors reflect

drinking categories: red and orange - very heavy and heavy drinkers, green and blue – binge and low drinkers.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1.

Attributes of alcohol self-administration recorded in individual monkeys.

Monkey ID	Group	PI	Intake (%)	Latency to Max Bout, s	Max Bout, ml*
_423	HD	50	53.5	3282	140.8
_581	HD	68	50.9	8098	149.9
_928	HD	150	43.5	7784	179.9
_606	HD	160	37.1	8162	173.5
_016	HD	192	32.8	17581	153.8
_547	HD	212	50.8	16445	108
Mean			44.8	10225	151.0
SE			3.4	2278	10.5
_492	NHD	173	38.5	607	116.6
_578	NHD	194	34.3	8344	89.2
_007	NHD	225	25.6	8402	75.7
Mean			32.8	5784	93.8
SE			3.8	2588	12.0

Each of the drinking parameters was recorded for all self-administration sessions. Median of the latency to maximal ethanol bout, median of the size of maximal ethanol bout and average daily ethanol intakes were used in this analysis.

* p<0.05 (Mann-Whitney test). HD and NHD are heavy and non-heavy drinkers, respectively.