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Behavioral Flexibility in alcohol drinking monkeys: the morning after

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Abstract

Background.—Heavy alcohol drinking has aspects of inflexible behavior. This study addressed the consequences of chronic alcohol drinking on cognitive and sensory-motor domains of behavioral flexibility in rhesus monkeys.

Methods.—Behavioral flexibility was assessed in 12 monkeys (n=9, ethanol drinkers) with a set-shifting visual discrimination procedure before alcohol self-administration and while maintaining consumption of 1.5g/kg/day ethanol. Task performance was assessed the morning after ~18 hours of drinking 1.5g/kg, and 1 hour before the next day's drinking session began. The first 10 set-shifting sessions had the original (pre-ethanol) test parameters and were used to determine retention of pre-ethanol performance. Then an effect of sensory-motor challenge (60% reduction in the size of the discriminative stimuli) on performance was assessed during 10 additional sessions.

Results.—There were no average group-dependent differences in the performance between control and ethanol groups at the pre-ethanol time-point. The daily consumption of 1.5g/kg/day produced binge alcohol intakes in 7 out of 9 monkeys (BEC ≥ 80mg/dl). Chronic daily intakes of 1.5g/kg had no effect on retention of the task in the sober state. However, when challenged with a reduction in the size of the stimuli, daily 1.5g/kg ethanol resulted in a decrement in performance due to an increase in the number of errors.

Conclusions.—Rhesus monkeys consuming 1.5g/kg alcohol daily perform equally as well as control monkeys in retention of a well-learned cognitive task. However, this pattern of daily alcohol intake robustly decreased the ability to flexibly adjust behavior when confronted with novel changes to perceptual stimuli.

Introduction

Crucial domains of behavioral flexibility include selecting proper actions, inhibiting inappropriate responses based on changing environmental demands (or rules) and

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Conflict of interests

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transferring these cognitive processes into an action. Thus, cognitive and sensory-motor domains interact to provide appropriate behavioral flexibility to the situation at hand. Heavy alcohol drinking has aspects of inflexible behavior, particularly under circumstances when an individual is unable to inhibit drinking alcohol to levels appropriate for situations that require relative sobriety or for avoiding adverse health consequences. Both acute and chronic alcohol intoxication have effects on cognitive and sensory-motor domains of behavioral flexibility. Cognitive aspects are primarily assessed in laboratory settings with response inhibition, set-shifting or task-switching procedures (e.g., Scaife and Duka 2009, Semenova 2012, Gass et al. 2014, Winward et al. 2014, Stock et al. 2016, Wolff et al. 2018, Zink et al. 2019) and sensory-motor domains are assessed with visual selectivity and hand-eye coordination tasks (Lex et al. 1988).

The Wisconsin Card Sorting Task and its analogues (e.g., ID/ED task) are widely used to measure cognitive flexibility across different species (Brown and Tait 2016). Recently, we developed a set-shifting task (SST) that is based on the human version of the card sorting task (Shnitko et al. 2017), with a unique feature of simultaneous testing in group of rhesus monkeys so that individuals can be rank-ordered on their ability to shift response patterns when reinforcement contingencies are altered. This SST is based on acquiring a series of discriminations between two stimuli that differed only on a single dimension (shape) or two dimensions (color and shape). Reversals of the correct stimulus were imposed after reaching criteria for acquisition of the discrimination, providing an opportunity to measure an individual's ability to alter their response strategies (i.e., demonstrate cognitive flexibility). Performance on the task predicted future heavy alcohol consumption in rhesus macaques (Shnitko et al. 2019), and suggests that a reduced ability to optimize behavior when contingencies change is a predisposing factor to heavy drinking. To extend these results, the present study is focused on the consequences of chronic alcohol drinking on behavioral flexibility in this same cohort of rhesus monkeys. Specifically, schedule-induced polydipsia procedure (SIP) is used to induce alcohol drinking in the monkeys and maintain self-administration of 0.5, 1.0 and 1.5 g/kg/day for 30 consecutive days at each dose (Grant et al. 2008). Drinking under the fixed-time schedule of food delivery generally produces rapid ethanol consumption in monkeys (within 2-hour period) that elevates blood ethanol concentration (BEC) above 80mg/dl when animals drink the 1.5 g/kg dose (equivalent of approximately 6 standard drinks in a 70 kg person). A pattern of alcohol intake that brings the BEC to 80mg/dl, or above, that typically occurs after consumption of 5 standard drinks in man within 2-hour period is defined by the National Institute on Alcohol Abuse and Alcoholism as a binge drinking (NIAAA 2004).

Cognitive and sensory-motor domains of behavioral flexibility were assessed with the SST. In this longitudinal design, first retention of the SST was measured (Shnitko et al. 2019) and then a sensory-motor challenge to the SST was assessed by decreasing the size of the visual stimuli. A similar approach has been used in human subjects where recognition of visual shapes was influenced by the size of the shapes presented (Jolicoeur 1987). The results show that daily alcohol intoxication, increasing from 2 to 6 drinks equivalent per day, did not impair retention of the average SST performance compared to not consuming alcohol when tested in the sober state. However, adjusting to a new sensory dimension of the task did attenuate behavioral flexibility in the alcohol drinkers.

Methods

Animals

Male rhesus monkeys (cohort 14, *Macaca mulatta,* n=12, 5-6 kg body weight) were obtained from the breeding colony of the Oregon National Primate Research Center and enrolled in the study at 3.5-4 years of age. An indoor housing room was kept at controlled temperature (20-22°C), humidity (65%), and an 11-h light cycle with lights on at 07:00 a.m. Individual monkeys were housed in metal cages (0.8 × 0.8 × 0.9 m) and were paired for 1 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 and delivery is described below (experimental procedures). All monkeys were weighed weekly in order to calculate ethanol doses, or maltose-dextrin solution for controls, based on their body weight (g/kg). 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 subject in the study had access to water, ethanol solution, food and the set-shift task through operant panels integrated into one of the walls of each housing cage (Vivian et al. 2001, Grant et al. 2008, Shnitko et al. 2017). In addition, controls received calorically matched 10% maltose-dextrin via a separate hanging bottle on the front of the cage. Each individual panel was connected to a panel's computer located outside the cage. Then all panels' computers were connected to a circuit and controlled from a main computer. The panels included a dowel located in a center, 2 drinking spouts on the right and left from the dowel with a food receptacle located below one of the spouts. The receptacle was connected to a 1 g pellet dispenser (Med Associates Inc., USA) and an infrared beam finger-poke situated below the receptacle. The spouts were connected to a plastic bottle placed on a digital scale (Ohaus Adventurer, USA) outside the cage. The bottles were refilled daily with either filtered tap water or 4% ethanol (w/v) diluted in water. A 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 for the water, ethanol and food intakes, as well as the running set-shift task, used a National Instruments interface and LabView software (LabView 2011, SP1, National Instruments, TX, USA).

Alcohol self-administration and blood ethanol concentrations

According to the experimental design in Figure 1, the alcohol self-administration began after the baseline set-shift performance was established (days 0 to 30). Twelve monkeys were assigned to experimental (ethanol, n=9) or control (water, n=3) groups and subjected to the SIP. As previously described (Vivian et al. 2001, Grant et al. 2008), polydipsia, or excessive drinking, is a form of adjunctive behavior that develops when food pellets are delivered to animals under a fixed-time (FT) schedule (Falk 1966). In this study, the banana pellets were delivered to monkeys under a FT schedule of 300 seconds. Figure 1 depicts an example of the schedule for an ethanol monkey in this study on a daily 16-hour session ("within daily schedule of events"). Each session included 3 distinct stages. At the beginning of the session

(*stage 1*) 1 gram pellets were delivered under FT300 and either water (control animals only) or 4% ethanol was available for drinking from one of the two spouts (i.e., the “induction” spout). During this stage the animals were required to consume the ethanol dose-based volume of either water or 4% ethanol (Figure 1 insert, blue bar, *stage 1*). Note that animals were not restricted on the amount of time to drink the required volume, but rather drinking was self-paced. After the required volume of fluid was consumed, the ethanol spout was closed and *stage 2* of the session began. During this stage the animals were given 2-hours of access to only water from the second, non-“induction” spout (Figure 1 insert, gray bars, *stage 2*). For the remainder of the session, access to water (through non-“induction” spout only) and the portion of daily food (pellets) were available until end of the 16 hour session (Figure 1 insert, brown bars, *stage 3*). Initially all monkeys were induced to drink water at the volume that would be equivalent to 1.5g/kg of ethanol in each daily session for 30 consecutive sessions (Figure 1, days 31-60, water induction). Next, water was replaced with 4% ethanol for the ethanol group. The required volume to drink under the FT300 schedule was adjusted to obtain daily doses of ethanol in 30-day increments: 0.5 g/kg (day 61-90), 1.0 g/kg (day 91-120) and 1.5 g/kg of ethanol (day 121-150).

All animals were trained to present their leg through an opening in the front wall of the housing cage and comply with venipuncture without the use of anesthesia. Every 4-5 days, a 20 ul blood sample for blood ethanol concentration (BEC) assay was collected from the saphenous vein at 30, 60, 90 min after the start of the SIP sessions during the 0.5 g/kg, 1.0 g/kg and 1.5 g/kg of ethanol sessions, respectively (Figure 1 insert, red overlay on blue bar). Then BECs were determined by gas chromatography (5890 Series II, Hewlett-Packard, Avondale, PA). The control animals (n=3) went through the same daily induction procedures as the alcohol drinking monkeys; however, only water was available through the “induction” spout and the blood sampling for BEC measurement was mimicked in them, but no actual samples were collected and processed.

Set-shifting task

The set-shifting task (SST) was used to explore behavioral flexibility in this group of 12 rhesus macaques prior to any ethanol exposure (at baseline, Shnitko et al. 2017). Similar to this earlier study, here all subjects were tested within their housing cages simultaneously, between 9:00 and 11:00 in the morning and before alcohol was available. The daily session provided up to 8 sets of 2 geometric shapes and colors presented on a computer screen. During each set the monkeys had to learn which shape is correct based on hit or miss strategy and alter behavior accordingly. Each pair of shapes and colors differed across sets unless the set was a reversal of the previous contingency (see Shnitko et al. 2017 for additional training details). A set was completed when the criterion of 12 correct trials out of 15 consecutive trials was met, and the next set began (with either the “correct” stimulus reversed within the same stimuli or a new pair of stimuli were presented). If a monkey met the criterion, it was presented with a reversal set, where the previously incorrect shape was now the correct one. In order, the sets of stimuli served for a unidimensional discrimination, two interdimensional discriminations and an extradimensional discrimination when color of the object became the correct dimension for the discrimination (extradimensional shift). The progression through the 8 possible sets in the daily sessions was self-paced, and the session

ended after 45 minutes had elapsed or when a monkey met the criteria for all 8 sets (i.e., under 45 minutes).

As shown in Figure 1, control and ethanol monkeys were tested on the SST in the morning prior to water and ethanol-induction procedure (30 sessions) and after they had completed 30 sessions of daily 1.5 g/kg ethanol induction. The 20 set-shift sessions during the induction of binge (1.5 g/kg) drinking were conducted in the morning prior to the ethanol induction sessions and ~18 hours after the previous day's ethanol intake. The 20 SST sessions were divided into 2 periods. During the first 10-sessions the parameters of the SST (timeout time, session time, order of the sets, time of preferred pictures presentation, reinforcement schedule, size of the discrimination stimuli and their location on the computer screen) were kept identical to the settings at the baseline, prior-ethanol test. This was used to assess the effect of chronic binge drinking on sober performance of a well-learned SST (i.e., retention). The ability to adapt to unexpected changes in a previously unaltered dimension of the SST stimuli was assessed with reducing size of the stimuli to 1/3 of original size (from 250px to 83px) with all other parameters intact.

Data analysis

The main dependent variables for each SST session were: the ratio of total number of errors to trials, the maximum set reached (out of the 8 possible sets) and the session duration (45 min or less). These three parameters were scaled from 0 to 100 and summed to obtain a performance index (PI) (Shnitko et al. 2017). The PIs obtained for each session were analyzed in a linear mixed model (LMM) regression analysis with random effect of subject and fixed effects of group (control, ethanol), period (prior to and after ethanol), stimuli size (250px and 83px), and session. Separate regression analyses of the ratio of errors to trials and session duration was performed. A one-way ANOVA with repeated measure (3 doses of ethanol) followed up by Bonferroni t-test was used to analyze BEC and time to finish a dose. A Spearman correlation analysis was used to estimate a relation between time to finish a dose and BEC. All analyses were conducted using SPSS 24 and $p<0.05$ were considered as significant. All data graphs were made using GraphPad Prism 7.0a version for Mac OS.

Results

Macaque model of binge alcohol drinking

Nine rhesus monkeys were induced to drink 4% (w/v) ethanol solution (diluted in water) using the SIP procedure (Grant et al. 2008, Baker et al. 2017). A representation of the cumulative records of ethanol intake from a single monkey are shown in a Supplemental Figure 1. Figure 2 shows the ethanol intake patterns, BECs and *stage 1* durations in these animals (time of the ethanol dose intake). The group BEC data (n=9) are shown in Figure 2, Column A. The induction dose of 0.5g/kg resulted in within-subject median BECs at 30 minutes into the session ranging from 0 and 48mg/dl; the dose of 1.0 g/kg resulted in within-subject median BEC at 60 minutes into the session ranging from 0 and 86mg/dl and the dose of 1.5 g/kg resulted in within-subject median BEC at 90 minutes ranging from 25 to 128mg/dl (average BEC: 87.9±13.6mg/dl). Overall, the BECs increased during the transition from 0.5g/kg to 1.5g/kg ($F_{2,24}=11.5$, $p<0.001$), and the average BEC for 1.5g/kg was

different from BEC at 0.5 and 1.0g/kg (post hoc test, both $p<0.05$, both $t>2.6$). The time to finish the required dose of ethanol was similar for the 0.5g/kg, 1.0 and 1.5 sessions ($F_{2,24}=0.8$, $p=0.4$, Figure 2, Column B). The times varied between 2 to 185 minutes during 0.5g/kg dose, 3 to 263 minutes during 1.0g/kg dose and 21 to 232 minutes during 1.5g/kg dose (average latency: 83.8±24.8 min). Overall, the median BEC was related to median time to finish the induction dose (*stage 1* duration) as revealed using Spearman correlation analysis (all $r<-0.79$, all $p<0.05$). As shown in the pie-charts in Figure 2, Column A, all 9 subjects were categorized as low drinkers at 0.5g/kg with all BECs recorded as < 80mg/dl. During the induction of 1.0g/kg, 5 monkeys had a BEC >80mg/dl at least once (categorized as bingers), with 1 of these categorized as a chronic binger due to BECs being consistently >80mg/dl (subject 8). The remaining 4 monkeys had BECs <80 mg/dl (low drinkers). During the induction of 1.5g/kg ethanol, 2 additional monkeys achieved the binge category (had a BEC >80mg/dl at least once); thus, a majority of monkeys (n=7/9) were categorized as binge drinkers due to at least 3 recorded BECs > 80mg/dl within the 6 blood samples/ subject over the 30 sessions of daily 1.5 g/kg. Five monkeys were categorized as chronic bingers due to BECs being consistently >80mg/dl.

Performance on the set-shift task.

As shown in Figure 3 performance on the set-shifting task was similar between the ethanol and control groups prior to ethanol exposure and 4 months later in 10 consecutive sessions (main parameters of the SST were kept identical to the settings at the baseline, Figure 3A,B). Specifically, the groups average performance gradually increased across the first 30 sessions of set-shift testing. The average PI increased from 106±25 during the first session to 205±9 on the 30^{th} session for the control animals and from 86±28 during the first session to 164±69 on the 30^{th} session for the ethanol animals (LMM analysis of baseline performance: effect of session, $F_{29,68}=1.7$, $p<0.05$; group, $F_{1,22}=1.3$, $p=0.3$; interaction, $F_{29,68}=1.3$, $p=0.2$). When under the daily induction of 1.5 g/kg ethanol, the average PI was not different from baseline performance in 50% of animals (1 control and 5 ethanol monkeys) and the PI was decreased in 50% of animals (2 controls and 4 ethanol monkeys). Statistical analysis did not reveal a significant effect of session [$F_{9,78}=1.6$, $p=0.1$], phase (10 sessions at baseline and 10 sessions after 4 months of induction) [$F_{1,81}=1$, $p=0.3$], group [$F_{1,17}=0.05$, $p=0.8$] or interactions (session x group [$F_{9,78}=0.7$, $p=0.7$], session x period [$F_{9,38}=1.2$, $p=0.3$], group x period [$F_{1,81}=0.8$, $p=0.4$], or session x group x period [$F_{9,38}=0.9$, $p=0.5$]).

Adaptation to decreasing the size of visual stimuli

While continuing under the daily induction of 1.5 g/kg ethanol (or water for control monkeys), an additional 10 sessions were evaluated wherein the size of the discriminative stimuli was decreased to 1/3 of the original size (Figure 3C). The average PI when using the original size of stimuli on session 40 was 196±11 in the control subjects and 183±58 in the ethanol drinkers. Set-shift sessions 41-50 used the size of the stimuli 5.5cm x 5.5cm (83px x 83px). As shown in Figure 3C, the smaller stimuli had a robust effect on the PI in both control and ethanol groups. The average PI decreased to 121±56 in the control group and to 84±28 in the ethanol group. There was a significant effect of session ($F_{(9,54)}=3.3$, $p<0.01$) and of group x stimuli size interaction ($F_{(1,120)}=22.2$, $p<0.00001$) with non-significant main effects of the group or stimuli size. To address the interaction between the effect of group

and stimuli size Bonferroni pairwise comparisons revealed decreasing the stimuli size was significant within each group of monkeys ($p<0.05$ for both groups). The PI was differentially impacted in the control and ethanol groups when presented with smaller shapes (i.e., $p=0.9$ in the group comparison with large shapes and $p<0.01$ in the groups comparison with smaller shapes). Nevertheless, the PI increased across the 10 sessions with the smaller shapes in each group. Individual subject PI gradually improved in both ethanol and control animals and by session 50 (the 10^{th} session with the smaller shape) average PIs reached 220±21 and 167±53 in the control and ethanol group, respectively.

As stated above a majority of monkeys (n=7/9) were categorized as binge drinkers with 5 of these categorized as chronic bingers due to BECs being consistently >80mg/dl. To compliment the analysis, we performed additional comparison of the task performance between the control and chronic binge drinkers (Supplemental figure 2). The analysis revealed a significant difference in performance between control animals and chronic binge drinkers when smaller stimuli were used during the test-sessions.

Effect of decreasing the stimuli size on individual components of the PI

The PI is a compound variable combining the session errors to trial ratio, the set reached, and the session duration. The introduction of smaller stimuli increased the proportion of errors made in the sessions in the ethanol group of animals (Figure 4A). The ratio of errors to number of trials was compared across 10 sessions before and after the decrease in the size of stimuli using mixed model linear regression analysis. There was a significant effect of session ($F_{(9,47)}=2.8$, $p<0.05$) and group x stimuli size interaction ($F_{(1,107)}=13.5$, $p<0.00001$) with all other main factors or interactions not significant (all $F<1.9$, all $p<0.05$). Bonferroni pairwise comparisons revealed that decreasing the stimuli size significantly increased errors/trial in the ethanol group ($p<0.0001$), but not in the control group ($p=0.06$). The average errors/trial for control subjects increased from 0.36±0.01 (large shape, session 40) to 0.41±0.04 (small shape, session 41) and then decreased to 0.32±0.04 (small shape, session 50), but this change was not significant (Figure 4C, one-way ANOVA, $p=0.16$, $F_{(2,6)}=2.5$). The average error/trial for ethanol subjects significantly increased from 0.34±0.03 (session 40) to 0.46±0.02 (session 41) and subsequently decreased to 0.36±0.02 (session 50) (Figure 4C, one-way ANOVA, $p<0.01$, $F_{(2,24)}=8.3$) A post hoc Bonferroni test revealed significant differences between sessions 40 and 41 ($t=3.7$, $p<0.01$) but not between sessions 40 and 50 ($t=0.5$, $p>0.9$).

The significant increase in the number of errors with the smaller shape size subsequently increased the session duration in ethanol groups of subjects (Figure 4B) primarily due to a 10 second timeout for an incorrect response as opposed to a 2 second intertrial interval following a correct response. A similar increase in session duration occurred in the control group. There were significant effects of session ($F_{(9,28)}=3.8$, $p<0.01$) and size of the stimuli ($F_{(1,89)}=4.3$, $p<0.05$), along with a significant interaction between treatment group and stimulus size ($F_{(1,89)}=18.9$, $p<0.00001$). Bonferroni pairwise comparisons revealed that the change of the stimuli size had a significant effect on session duration within each group of monkeys ($p<0.0001$ for control and $p<0.05$ for ethanol). The average session duration in control animals rose from 32±4 minutes for session 40 to 45 minutes immediately after the

decrease in stimuli size, and then robustly decreased to 23±5 minutes (session 50, Figure 4D, one-way ANOVA, p=0.002, $F_{(2,6)}$=19.6). In the ethanol group, the average session duration on session 40 (prior to decreasing stimuli-size) was 32±11 seconds and increased to 45 minutes in the first session of smaller stimuli (session 41, Figure 4D, one-way ANOVA, p=0.02, $F_{(2,24)}$=4.9). As opposed to the controls, the ethanol group showed less improvement across 10 sessions and finished with an average session duration of 37±11 min in session 50.

The increase in the number of errors with the smaller stimuli size decreased animals' progression through the sets within each session. Thus, the majority of control and ethanol drinking monkeys reached the 8th set (reversal to extradimensional shift) when presented with the original (large) stimuli. This parameter of the task performance dropped to set 3 (intradimensional shift) in ethanol and control animals immediately after the decrease in stimuli size (session 41). In the control group, animals were able to reach reversal to extradimensional shift (set 8) by session 42 and maintained this for the rest of the testing. In contrast the majority of ethanol drinkers were consistently reaching set 8 in session 48.

Discussion

This study used a SIP procedure to reliably induce chronic alcohol drinking in a group of young adult rhesus monkeys (Grant et al. 2008) while being tested for impairments in their ability to retain performance on a SST given in the sober state (Shnitko et al. 2017). The SIP procedure allowed for identifying individual differences in the patterns of ethanol intake (latency of ethanol intake and BEC) leading to categorizing monkeys as low, binge or chronic binge drinkers based on a definition of binge drinking as a pattern of alcohol intake that brings the BEC to 80mg/dl or above after consumption of 5 standard drinks (in men) within a 2-hour period (NIAAA 2004). In this study 80% of subjects exhibited binge drinking under the induction dose of 1.5g/kg, with a BEC >80 mg% detected in these subjects at least 3 times within a 30-day period. Moreover, the average individual BECs were negatively correlated with the average time to drink 1.5 g/kg ethanol. Further, an alcohol drinking pattern of 5 or more bingers within a month is considered heavy drinking (SAMHSA 2018). Under this definition, 5 out of 9 monkeys could be categorized as heavy bingers. Thus, the SIP procedure is an effective method to model binge drinking while revealing individual differences in the drinking patterns in macaque monkeys. Similarly, Hosová and Spear (2017) found that SIP can also model binge-like ethanol intake in an adolescent rodent model of self-administration (Hosova and Spear 2017).

To our knowledge this is the first study in laboratory animals that used a longitudinal design to assess effects of chronic alcohol self-administration on the cognitive and sensory-motor domains of behavioral flexibility. The majority of previous studies that explored alcohol effects on cognitive flexibility were done with rodents, using a cross-sectional study design and demonstrated that ethanol exposure (vapor cambers, intragastric injections) attenuates cognitive flexibility (e.g., Gass et al. 2014, Sey et al. 2019). Additionally, in nonhuman primate models of alcohol drinking, chronic alcohol attenuated major executive functions supporting behavioral flexibility, such as working memory, visual discrimination, response time and accuracy (Taffe et al. 2010, Crean et al. 2011, Wright and Taffe 2014, Chandler et al. 2017). In this study, following the chronic alcohol consumption monkeys, as a group, had

nearly identical performance as a control group on the set-shift task assessed in a sober condition when the testing parameters were the same as before alcohol drinking (Figure 3B). It appears that pre-ethanol experience with the task allowed retention of knowing the basis of the sequential discriminations. Daily chronic episodes of alcohol binge drinking did not impair the ability to perform under the well-learned and anticipated rules of the set-shifting task when tested in the sober state. Studies in human subjects, where cognitive measures were collected longitudinally at baseline and at a follow up experiment, showed that prior experience with the test (e.g. baseline measures) strongly enhanced the test performance during the follow up experiment (Salthouse 2015, Sullivan et al. 2017). A recent study by Schreiner and Gremel (2018) showed that a larger amount of experience with a known task rule predicts less flexibility when a novel task rule is introduced in mice (Schreiner and Gremel 2018). In the present study with rhesus monkeys, the daily experience with the SST led to the development of highly skilled or automatic performance of the task allowing for a greater efficiency of the behavior (Tiffany 1990). Transitioning to performing with a new stimulus dimension led to an immediate and significant decrease in performance observed in both control and ethanol groups (Figure 3C). The performance decrement was evidenced by an increased latency to touch a stimulus and the number of touches outside of the stimuli location (supplemental Figure 3). However, the performance of alcohol drinking monkeys showed greater initial deficits compared to the controls when challenged with the reduction in the size of the stimuli, and this deficit remained over the next 10 testing sessions. Thus, a history of chronic alcohol drinking amplified the sensitivity of established behavioral strategy used during the task performance for the change in stimuli size leading to more errors and longer sessions (Figure 4). Importantly, the control subjects had a trend for an initial increase in errors ($p=0.06$) when the stimuli size was decreased and further study with larger number of control subjects will be necessary. There is a large number of studies showing that chronic alcohol consumption shifts behavioral control from a flexible, goal-directed, model-based actions toward inflexible, habitual, model-free behaviors (Sebold et al. 2014, McKim et al. 2016a, McKim et al. 2016b). In this study, chronic alcohol impaired a flexible adaptation to the change in a novel visual dimension compared to control animals.

As a process, behavioral flexibility is an ability to adjust behavior according to changes in learned associations. This executive function relies on functional associative neurocircuitry including the dorsolateral prefrontal cortex and subcortical caudate nucleus (Mansouri and Buckley 2018). Excessive alcohol drinking is thought to weaken the dependence on associative neurocircuitry in favor of sensorimotor circuits and attenuating flexible behaviors (McKim et al. 2016). In this study, monkeys assessed in the mornings following chronic alcohol binge drinking showed reduced sensory perception when the dimension of visual stimuli was altered and adapting a new strategy for touching the stimuli. Future studies can address if greater than 6 drinks/day can impair the cognitive component of performance flexibility, as over 50% of this population of rhesus monkeys showed much greater intakes of alcohol if allowed (Grant et al. 2008, Baker et al. 2014). Further, higher average daily intakes (i.e., >20% of daily intake over 3.0 g/kg) alter striatal synaptic output consistent with increased habitual behavior (Cuzon Carlson et al. 2018). Overall, linking chronic heavy alcohol consumption, striatal synaptic adaptations and sensory, as well as cognitive aspects

of habitual behavior, provides a rich model for understanding the neural circuitry involved in alcohol use disorder.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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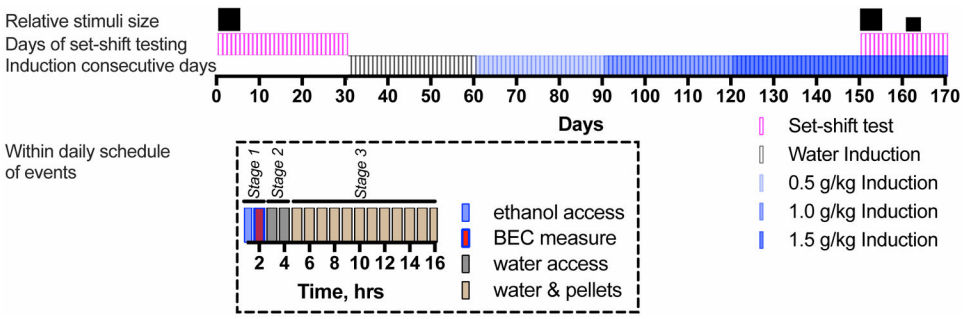

Figure 1.

Schematic representation of the experimental design. The set-shift testing was conducted prior to (30 sessions) and after alcohol self-administration (20 sessions). For the last 10 sessions, the size of the discriminative stimuli was reduced by 2/3 (from 250px to 83px) as indicated with a smaller black square on the top of the schematic (relative stimulus size). Induction procedure begins with water (30 days) then ethanol solution (4%, w/v) was introduced to monkeys. Monkeys were required to drink the dose of 0.5 g/kg/d (30 days, 2 standard drinks a day), then 1.0 g/kg/d (30 days, 4 standard drinks a day) followed by 1.5 g/kg/d (30 days, 6 standard drinks a day). Insert depicted by dashed-line rectangle: Representation of a single random day during induction with 1.5 g/kg/d. An induction session is 16 hours per day. From the beginning, animals have access to 4% ethanol solution (or water) and banana flavored pellets are delivered to them on a FT300 schedule (blue bars, *stage 1*). Animals are required to drink the ethanol solution at a volume sufficient to obtain 1.5 g/kg/d dose (or other dependent on induction phase). A blood sample is collected 90 minutes after the session start for analysis of BEC (see methods for details on BEC analysis and collection). Immediately upon consuming the ethanol dose (time of intake might vary between animals within a single session and between sessions within a single animals), access to ethanol is terminated and only water is available for 2 hours (grey bars, *stage 2*). After 2 hours the remainder of the daily food ration and water are available until the end of the session (brown bars, *stage 3*).

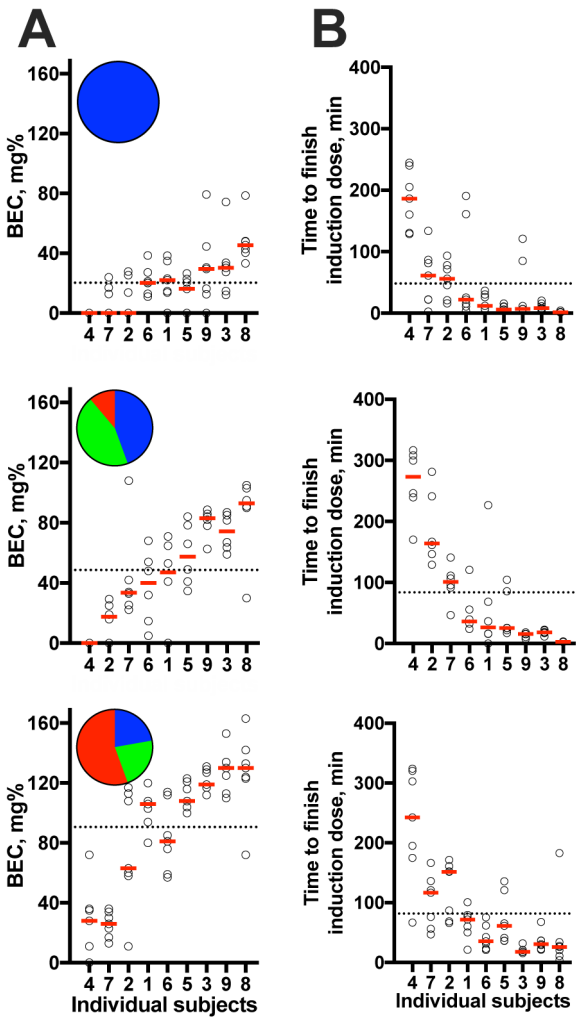

Figure 2.

Characteristics of alcohol drinking in the macaque model of schedule-induced alcohol self-administration. **Column A**: BECs (open circles) for each ethanol monkey plotted per induction dose (from 0.5g/kg/session on the top to 1.5g/kg/session on the bottom). For each induction dose, the average group BEC (n=9) is depicted by the dotted black horizontal lines, the median within-subject BEC is depicted by the short red lines. The inserted pie-charts show the proportion of monkeys that were categorized as low (blue), binge (green) or chronic binge (red) alcohol drinkers based on attaining a BEC of 80 mg/dl or above. **Column B**: Latency to consume the required dose (open circles) for each monkey plotted per induction dose (same as in B). Similar to the Column A, the dotted black lines depict the average group latency, the short red lines above each monkey depict within-subject median latency.

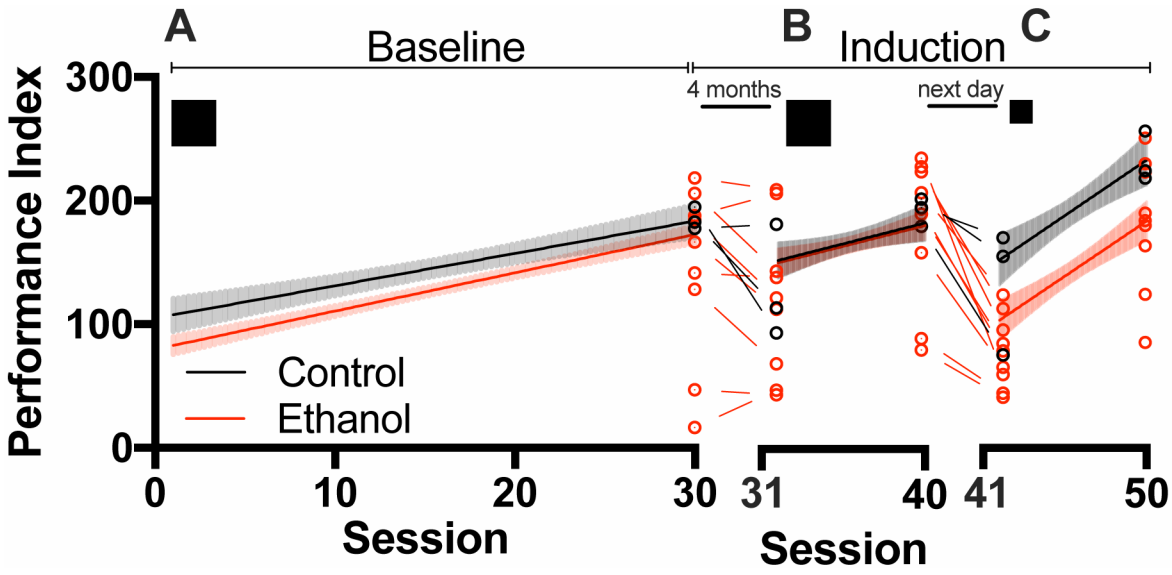

Figure 3.

Average performance for control (n=3) and ethanol (n=9) monkeys on the set shifting task across the 30 sessions of baseline and during the induction of water (controls) or 1.5 g/kg/day ethanol. Graphed are the groups performance across the 30 sessions prior to ethanol self-administration (A) and the next 20 sessions (sessions 31-40 (B) and 41-50 (C)) which occurred 4 months later and in the mornings prior to1.5 g/kg/day ethanol induction (or water for the control group). The individual circles depict within-subject performance indexes: (A) at baseline (10 session mean), (B) at the 1st and the last set-shift session during induction with the original size of the stimuli and (C) at the 1st and the last set-shift session during induction when the size of stimuli was reduced. The shaded areas in both graphs depict 95% confidence intervals.

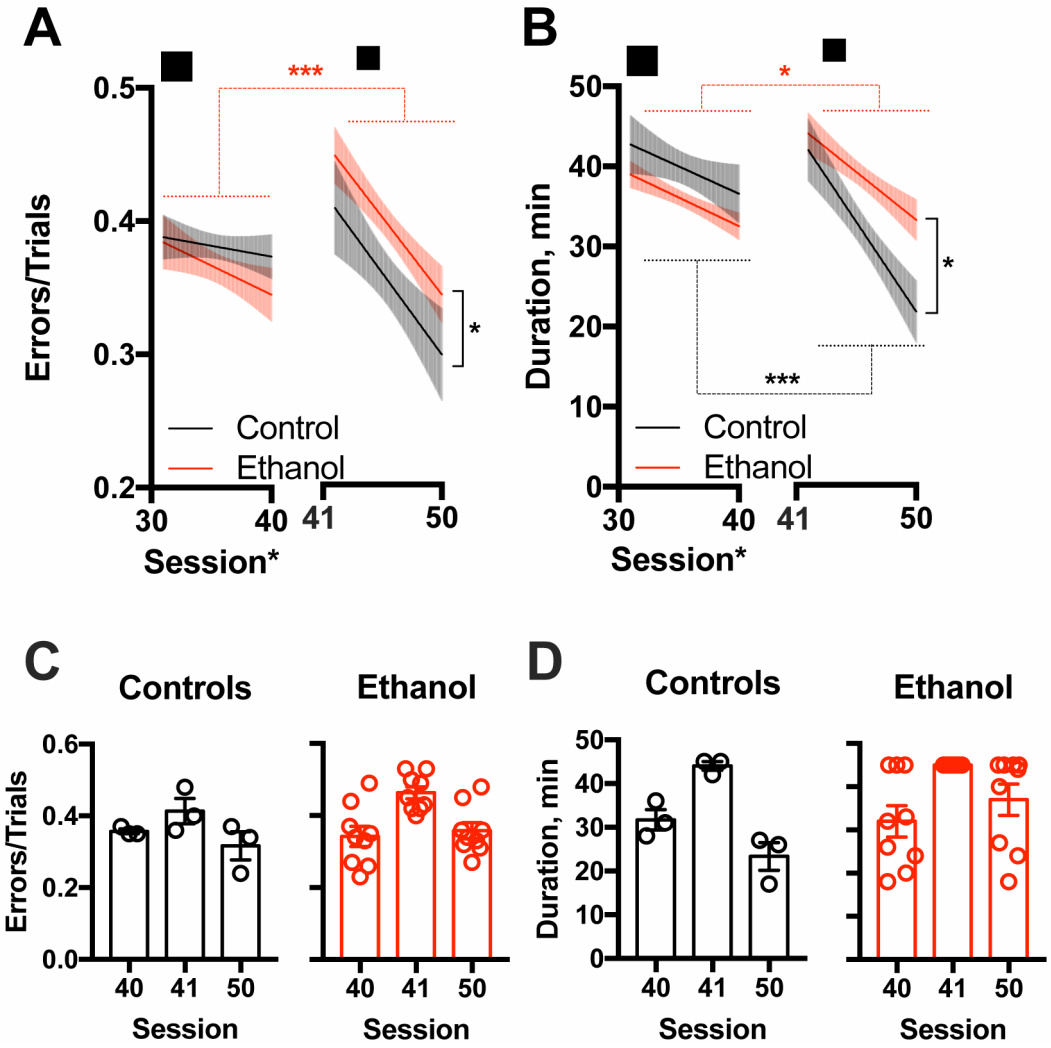

Figure 4.

The effect of decreasing the stimuli size on the task variables: ratio of errors to number of trials and session duration in consecutive set-shifting sessions during induction of 1.5 g/kg ethanol (controls were given water). A. Average number of errors per trial; red dashed line and corresponding *** depict significant increase in errors/trial within the ethanol group only ($p<0.0001$); * indicates significant group difference with the smaller shape across the 10 sessions. B. Average session duration with the larger and smaller size shapes. The maximum possible session time was preset to 45 minutes; however, monkeys could finish in less time depending on their progression through all 8 sets of the task. In this graph *** and * reflect significant differences within and between groups with p values <0.001 and 0.05, respectively. C and D. An additional analysis of the set-shift parameters measured in two groups of monkeys during session 40 (last set-shift sessions given with stimuli at their original size as at baseline), session 41 (the 1st session when stimuli size was reduced to 1/3) and session 50 (the 10th set-shift session with reduced size of the stimuli). * reflects significant differences within the groups with p values < 0.05 (vs session 40).