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ORIGINAL INVESTIGATION

Aggressive temperament predicts ethanol self-administration in late adolescent male and female rhesus macaques

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Abstract
Rationale Anxiety and aggression are associated with ethanol self-administration, but these behaviors can serve as either risk factors for or consequences of heavy drinking in rodents and humans. Baseline levels of aggressive-like and anxious-like behavior in non-human primates have not yet been characterized in relation to future or prior ethanol intake.
Objective The objective of the study was to test the association between temperament at baseline with future ethanol self-administration in late adolescent male (n = 21) and female (n = 11) rhesus monkeys.
Methods Shortly after entering the laboratory and before exposure to ethanol, the Human Intruder Test (HIT) and the Novel Object Test (NOT) were used to determine baseline anxious-like and aggressive-like behavior in age-matched male and female rhesus monkeys (*Macaca mulatta*). The monkeys were induced to drink ethanol 4 % (*w*/*v*) using a schedule-induced polydipsia procedure, followed by "open-access" ethanol self-administration in which the monkeys were allowed a choice of water or 4 % ethanol (*w*/*v*) for 22 h/day for 52 weeks.
Results Aggressive monkeys self-administered more ethanol and attained higher blood ethanol concentrations (BECs). No significant differences in ethanol intakes or BECs were found between anxious and non-anxious monkeys or between behaviorally inhibited and non-inhibited monkeys. Baseline aggressive behavior positively correlated with ethanol intake and intoxication.
Conclusions Baseline reactive aggression was associated with higher future ethanol intake and intoxication. While significant sex differences in HIT reactivity were observed, the relationship between aggression and ethanol drinking was observed across sex and is not sex-specific.

Keywords Temperament · Anxiety · Aggression · Risk factors · Sex differences · Monkey

Introduction

In the USA, 64 % of adults consume alcohol, but only 8 % meet criteria for alcohol abuse (Grant et al. 2004). As alcohol abuse and alcoholism constitute the third major preventable cause of death in the USA behind smoking and obesity (Mokdad et al. 2004), understanding individual risk for alcohol abuse is crucial. Many risk factors have been previously identified in human and monkey populations, including sex, availability, age of onset, anxiety and stress, and temperament (Barr et al. 2003; Barr and Goldman 2006; Gordon 2002; Grant et al. 2008a; Conner et al. 2010). Though studies of human alcoholics have identified variables associated with heavy drinking, the lack of longitudinal data and inconstant life histories of ethanol consumption and stress complicate the results. In contrast, controlled animal models, and non-human primates in particular, can play a key role in understanding the risk factors contributing to heavy drinking. Non-human

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primates absorb and metabolize ethanol at similar rates as humans, exhibit dimensions of temperament, experience complex social and affective processes, and chronically self-administer ethanol resulting in intoxication and physical dependence (Grant and Bennett 2003; Grant et al. 2008a).

Temperament, defined as a collection of individually variable emotional and behavioral reactions with temporal and situational stability (Kagan 1994), is an endophenotype for drinking to dependence that can be measured in both human and monkey subjects. An individual's genetic propensity toward the development of alcoholism may be partially expressed via temperament (Chartier et al. 2010, review), and in human subjects, temperament can predict adolescent alcohol use (Dick et al. 2013). Two of the most widely used and standardized non-human primate tests of temperament include the Human Intruder Test (HIT) and the Novel Object Test (NOT). Temperament tests in monkeys have primarily been used to assess behavioral inhibition and defensive behaviors including both anxious and aggressive responses. These defensive responses are characterized by reactive anxiety and/or reactive aggression in response to the direct stare from the human intruder in the form of behaviors such as teeth grinds, yawns, and threats.

In human subjects, externalizing disorders with aggressive components such as conduct disorder and oppositional defiant disorder have been found to predict or associate with substance use in adolescence (Disney et al. 1999; Boyle et al. 1999; Pardini et al. 2007; Fergusson et al. 2007), even when controlling for anxiety/depression and a family history of alcoholism (Jester et al. 2008). In addition, aggression outside of the diagnosis of a specific disorder may also relate to future alcohol abuse, with aggression at ages 5–10 increasing the odds of adolescent alcohol abuse (Brook et al. 1992) and aggression at first grade indirectly associating with substance use problems (Fothergill and Ensminger 2005). There are well-known associations between difficult temperament, antisocial personality, unstable temperament, and alcohol abuse (DeJong et al. 1993; Kessler 2004; Skodol et al. 1999). However, the direction of the association between aggression and alcohol consumption is unclear, with chronic alcohol problems associating with violence empirically in many populations and alcohol acting as a factor in 57–79 % of violent crimes (Mayfield 1976; Virkkunen 1974). Additionally, these associations do not specify a relationship with reactive versus controlled-instrumental aggression, with reactive aggression being more impulsive and controlled-instrumental aggression being more goal-oriented (Vitiello and Stoff 1997).

Similarly, the high degree of comorbidity between anxiety disorders and alcoholism in humans (Kushner et al. 2000) suggests an association, though the direction of the association is again unclear. Alcohol has anxiolytic potential, and higher levels of anxiety have been proposed to lead to higher levels of alcohol intake. For example, peer-reared rhesus monkeys that were separated from their mothers early in life and raised in a nursery environment displayed more anxiety-related behaviors and drank more flavored alcohol solution than monkeys raised by their mothers (Higley et al. 1991). On the other hand, chronic daily exposure to a low dose of ethanol (0.5 g/kg/day) increased anxiety and aggression in socially housed female cynomolgus monkeys (Shively et al. 2002). In human children, behavioral inhibition is characterized by extreme shyness and fearfulness and is predictive of future anxiety disorder development (Biederman et al. 2001; Caspi and Silva 1995; Svihra and Katzman 2004, review). Conversely, behavioral undercontrol is characterized by aggression, impulsivity, irritability, difficulty in state control, and a lack of persistence. A study by Caspi et al. (1996) following 1000 New Zealand children found both behavioral inhibition and behavioral undercontrol to be predictive of future alcohol problems in the male but not female subjects.

The current study used late adolescent male and female monkeys to assess the possibility of temperament acting as a risk factor for heavy ethanol intake by measuring associations between baseline measures of anxiety and aggression and future self-administration of ethanol. Based on previous studies, we hypothesized that monkeys with higher levels of anxiety or aggression at baseline would self-administer higher levels of ethanol when compared to monkeys with lower levels of anxiety or aggression.

Materials and methods

Animals

The 32 subjects included two cohorts of female (cohort 6A $n = 6$, age 3 years 10 months–4 years 1 month, weight 4.0–6.3 kg; cohort 6B $n = 5$, age 5 years 7 months–6 years, weight 5.0–6.2 kg) and three cohorts of male (cohort 7A $n = 8$, age 3 years 11 months–4 years 7 months, weight 5.5–7.5 kg; cohort 7B $n = 5$, age 5 years 7 months–6 years 3 months, weight 7.0–11.7 kg; cohort 10 $n = 8$, age 4 years 7 months–6 years, weight 6.5–9.8 kg) rhesus monkeys (*Macaca mulatta*; born and raised at the Oregon National Primate Research Center, Beaverton, OR). Ages indicate age at first drink of ethanol (first day of ethanol induction—see Ethanol access section), with all animals falling between approximately 4 and 6 years of age. Cohort nomenclature (6A, 6B, 7A, 7B, 10) is used to provide links to the drinking data through www.matrr.com. Monkeys were reared with their mothers in a troop until at least 2 years of age, after which they were moved to smaller group housing in single sex groups with their peers. All subjects were experimentally naïve at the onset of the study. Monkeys within each cohort had no common parents or grandparents. All monkeys were housed in individual cages with partitions allowing visual, auditory, and olfactory but non-physical contact to neighboring monkeys (0.8 × 0.8 × 0.9 m). Each individual cage contained an operant panel on one wall of the cage dispensing food and liquids. The housing room was maintained at a constant temperature (20–22 °C) and humidity (65 %) and a 12-h light cycle (lights on at 7:00 am). Body weights were taken weekly. Following acclimation to the laboratory, the monkeys were trained to participate in awake (non-anesthetized) venipuncture to obtain blood samples (Porcu et al. 2006) to assess blood ethanol concentration. All animal procedures were approved by the Oregon National Primate Research Center IACUC and were performed in accordance with the NIH and the *Guide for the Care and Use of Laboratory Animals.*

Temperament classification and behavioral testing

Anxious-like and aggressive-like behavior and temperament were measured via video recordings of two behavioral tests: HIT and NOT (Fig. 1). These tests are commonly used to assess anxious, fearful, defensive aggressive, and inhibited behavior in human children and monkeys (Fairbanks and Jorgensen 2011). Temperament testing of all monkeys occurred within 7 months of entry to the laboratory between 9:00 am and 1:00 pm (Table 1). Testing occurred in a novel individual testing cage in a behavioral suite physically separate from the laboratory and was video recorded from an anteroom through a one-way mirror. Test cages were cleaned after each monkey, and male and female subjects were never tested in the same cage or on the same day. One male rhesus cohort (cohort 10) was not tested with the NOT due to previous cohort data (see Results section). Video recordings of the HIT and NOT were scored by two observers unfamiliar with the monkeys and unaware of future alcohol consumption using the Observer XT software (Noldus Information Technology, Waegningen, Netherlands). Interrater reliability between the two observers was found to be very high ($\kappa = 0.81$, percentage of agreements = 84.5 %).

Human Intruder Test

The HIT reliably assesses individual differences in stress reactivity via three specific stimuli (Williamson et al. 2003). As shown in Fig. 1, testing began with a 10-min acclimation period and a 2-min control period during which the monkey was free to explore the testing cage in the absence of any other stimuli. During the 2-min profile period, the unfamiliar human “intruder” entered the testing room and stood 0.3 m away from the cage in profile to the monkey. A second 2-min control period followed the exit of the human intruder from the room and preceded the beginning of the stare phase. In this phase, the same human intruder entered the room and made continuous direct eye contact with the monkey for 2 min before exiting. Behaviors scored during this test include movements, vocalizations, exploration, and other reactions to the human intruder as listed in the ethogram (Table 2).

Novel Object Test

The NOT is used to examine responses to threatening and non-threatening novel stimuli. It has been applied to many different species (Belzung and Le Pape 1994; Kim et al. 2005), and longer latencies to approach novel objects during adolescence in humans are associated with the development of anxiety disorders (Garcia-Coll et al. 1984; Schwartz et al. 1999). Latency to inspect novel objects has also been shown to be heritable and stable over time (Williamson et al. 2003). The NOT began after the third control period of the HIT and consisted of 5-min presentations of three novel objects. Each object was brought into the room and removed by the human intruder. The novel objects included a kiwi (novel fruit), Mr. Potato Head (potentially threatening novel object simulating the stare of the HIT), and a rubber snake (threatening novel object) presented with a piece of apple (preferred food).

Temperament variable organization

Variables assessed during the HIT and NOT are found in Table 2. Durations of threat, partial threat, cage slap, and cage shake during the stare epoch were summed to create a new variable labeled “extreme threat.” Durations of freeze non-vigilant and freeze vigilant during the profile epoch were summed to create a new variable “freeze profile,” and durations of freeze non-vigilant and freeze vigilant during the stare epoch were summed to create a new variable “freeze stare.” Durations of teeth grind and yawn during the stare were summed to create a new variable “active anxiety.” Grouping of these variables was based on single linkage cluster analysis (joining) measuring Euclidian distances (Supplemental Fig. 1), kappa statistic calculations (Supplemental Tables 1 and 2), and Spearman’s correlations (data not shown).

Fig. 1 HIT and NOT testing protocol

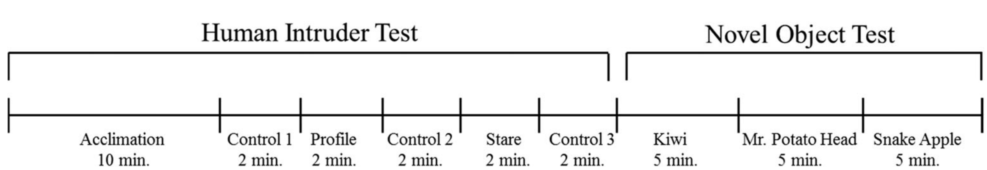

Table 1 Experimental timeline (weeks) by cohort

Rhesus monkey cohorts	Cohort 6A: females ($n = 6$)	Cohort 7A: males ($n = 8$)	Cohort 6B: females ($n = 5$)	Cohort 7B: males ($n = 5$)	Cohort 10: males ($n = 8$)
Acclimation	1–8	1–8	1–5	1–8	1–5
Temperament testing	14	23	6	27	6
Induction of self-administration (SIP)	41–52	24–38	103–120	40–58	23–43
22 h/day ethanol access	56–107	43–94	126–178	62–114	49–101

Temperament categorization

Responses to the human intruder were used to characterize monkeys as aggressive or non-aggressive, active anxious or non-active anxious, and inhibited or non-inhibited. Monkeys were characterized as aggressive if they displayed an extreme threatening behavior of any duration (threat, partial threat, cage slap, or cage shake) or if the duration of other aggressive behavior (open mouth threat) was at least one standard deviation above the mean of all subjects of their sex. All defensive aggressive behaviors elicited by the HIT were reactive aggressive behaviors. Monkeys were characterized as active anxious if the duration of reactive anxious behavior (yawning and/or teeth grinding) was at least one standard deviation above the mean of all subjects of their sex. Monkeys were characterized as inhibited if the duration of freezing in the stare or profile period was at least one standard deviation above the mean of all subjects of their sex. Responses to the novel objects were also used to characterize behavioral inhibition. The latencies in seconds to inspect, touch, and manipulate each novel object were measured, and monkeys were considered inhibited if their latency to interact with the novel objects was on average at least one standard deviation above the group mean of all subjects of their sex.

Ethanol access

As indicated in Table 1, a schedule-induced polydipsia (SIP) procedure was used to induce ethanol self-administration (Grant et al. 2008a). Timing of the onset of induction following temperament testing varied due to schedule constraints. Briefly, the scheduled delivery of 1-g food pellets every 5 min (fixed-time 5 min (FT-5 min)) was used to induce rapid intake of an available fluid. During SIP induction, the monkeys were subjected to the FT-5 min schedule of pellet delivery until a specified volume of water or 4 % ethanol (*w*/*v*, in water) was consumed. Every 30 days, the dose of ethanol consumed was increased from 0 g/kg/day (water volume equivalent to 1.5 g/kg ethanol) to 0.5, 1.0, and finally to 1.5 g/kg/day. The monkeys were allowed up to 16 h to drink the specified volume of water or ethanol but normally finished between 5 min and 3 h (see Grant et al. 2008a for additional details). Following the 120 sessions of induction, "open-access" self-administration began and ethanol (4 % *w*/*v*) and water were concurrently available for 22 h/day, 12 pm–10 am. Monkeys with a daily average greater than 3.0 g/kg were defined as heavy drinkers (Grant et al. 2008a), and monkeys with intakes below this threshold were defined as non-heavy drinkers.

Self-administration equipment

Within each monkey's home cage, a drinking panel on one wall permitted access to all fluid and food. Drinking panels were controlled via a computerized system (Macintosh G4, Apple Computer Inc., Cupertino, CA, with National Instruments hardware and programming environment, National Instruments Corporation, Austin, TX). Each panel contained two drinking spouts: a set of three lights (red, white, and green) located below one of the spouts and a centrally located opening containing a dowel with an associated stimulus light. Each spout was connected by tubing to a 1–l fluid reservoir and placed on a digital scale (Ohaus Navigator Balances N1B110, Ohaus Corporation, Pine Brook, NJ) interfaced to the computer system. Drinking volumes and patterns were acquired by using serial communication to retrieve changes in the weight of the fluid reservoirs.

Assays

Blood draws (3 ml) provided the plasma to be used for future assays. Blood ethanol concentrations (BECs) were measured using 20 μl of whole blood and headspace gas chromatography approximately every fifth day 7 h into the 22 h/day drinking session, resulting in approximately 66 observations per monkey during 12 months of ethanol self-administration.

Statistical analysis

Prior to all analyses, distributional assumptions were tested. Independent variables included sex, temperament status at baseline (groups determined as described above), and durations of anxious-like and aggressive-like behaviors at baseline. Dependent variables included daily ethanol intake (g/kg/day), daily water intake (g/kg/day), and BEC (mg%) averaged across 12 months of 22-h access to ethanol. Sex differences in the dependent variables were assessed with independent two-sample t tests or Welch's t tests, while sex differences in behavioral responses from the HIT and NOT were assessed with Mann-Whitney U tests. All other statistical analyses were performed across sex. Group differences in ethanol self-administration and intoxication based on baseline

Table 2 Behavioral ethogram for temperament testing

Behavior[a]	Category[b]	Definition
Stationary	–	Subject is inactive without motile movement; may still involve head or arm movement
Locomote	–	Subject engages in movement from one location to another while using its entire body
Movement	–	Subject engages in movement of body but does not change position in cage
Freeze	Inhibition	Subject is not engaged in any movement of body or head
Explore	–	Subject inspects or manipulates cage
Sleep	–	Subject is inactive with eyes closed
Cage bite	–	Subject uses mouth to grasp bars of cage
Self-groom	–	Subject is picking through and/or slowly brushing aside own fur with hands and/or mouth
Abnormal	–	Subject is engaged in atypical behavior; may include any of the following: *self-bite, copraphagy, floating limb*
Stereotypy	–	Subject paces back and forth or is engaged in other repetitive motion
Other	–	Subject is engaged in behavior not listed in ethogram
HIT specific behavior		
Coo	Anxiety	A short, high pitched soft vocalization
Shriek	Aggression	A very high pitched loud vocalization
Grunt	–	A short, low pitched vocalization
Bark	Aggression	A very short, loud vocalization
Other vocal	–	Any other vocalization
Yawn	Anxiety	Subject opens mouth very wide, baring upper teeth
Scratching	Anxiety	Subject uses fast movement of the hand or foot across the hair or skin
Cage slap	Aggression	Slapping the floor of the cage with hands
Teeth grind	Anxiety	Subject engages in audible side to side movement of jaws with teeth rubbing together; usually directed at stranger
Lipsmack	–	Quick movement of jaw pressing lips together; usually directed at stranger
Threat	Aggression	Subject stares intensely with eyes wide open and/or ears pulled back; may contain facial, vocal, or physical components (e.g., *head thrusting, open mouth threat, scream, raised eyebrow, ground beating, lunge*); usually directed at stranger
Open mouth	Aggression	Subject opens mouth in "o" shape, may be accompanied with thrusting head; usually directed at stranger
Cage shake	Aggression	Subject uses hands and/or body to attempt to move cage back and forth
Partial threat	Aggression	Behavior that appears threatening but does not fall into one of the other categories; may include slight lunge or charge directed at stranger
Fear grimace	Anxiety	Subject has lips pulled back bearing teeth, in a "smile"; usually in response to stranger
No response	–	Subject is not engaged in any behavior directed toward stranger, or stranger is not present
NOT specific behavior		
Inspect	Inhibition	Subject responds to novel food or toy by looking at or coming toward it very closely and/or sniffing
Touch	Inhibition	Subject responds to novel food or toy by using hands and/or lips without moving the object
Mouth	Inhibition	Subject puts object in mouth but does not eat
Manipulate	Inhibition	Subject responds to novel food or toy by moving its location

HIT Human Intruder Test, *NOT* Novel Object Test, – the behavior was not used to characterize temperament

[a] State behaviors within a behavioral class are mutually exclusive and exhaustive

[b] Indicates which behaviors were used to characterize temperament

temperament (aggressive versus non-aggressive, etc.) were conducted with independent two-sample t tests or Welch's t tests. Multiple group comparisons were corrected with the Bonferroni correction. Correlations between independent and dependent variables were analyzed with Spearman's rank-order correlations. Correlations driven by a single data point were omitted. In accordance with published recommendations, correlational analyses were not adjusted for multiple comparisons due to the preliminary nature of this study and small sample size (Perneger 1998; Rothman 1990). All analyses were conducted by using Statistica Academic with alpha values considered significant at $p \leq 0.05$.

Results

Responses to temperament tests

Aggressive behavior was observed in 9 out of 11 female monkeys and 6 out of 21 male monkeys. Based on the aggressive behaviors exhibited during the HIT, 4 out of 11 female monkeys and 4 out of 21 male monkeys reached criteria for aggressive temperament. Based on the active anxious behaviors exhibited during the HIT, 3 of the 11 female monkeys and 2 of the 21 male monkeys were characterized as anxious. Responses to the NOT characterized 5 of the 11 female monkeys and 8 of the 21 male monkeys as behaviorally inhibited, whereas responses to the HIT characterized 3 of the 11 female monkeys and 4 of the 21 male monkeys as behaviorally inhibited (Table 3).

Relationship between baseline temperament and ethanol self-administration during 22-h access

Aggressive monkeys self-administered significantly more ethanol (3.65 versus 2.34 g/kg/day, $t_{30} = 3.5$, corrected $p = 0.004$,

Table 3 Average daily intake of ethanol (4 % *w/v*) and BEC during 22-h ethanol access and anxious/aggressive status at baseline

Sex	Cohort	Monkey	12-Month intake (g/kg/day)	12-Month BEC (mg%)	HIT aggressive	HIT active anxious	HIT inhibited	NOT inhibited
Female	6A	97[a]	5.1	99.3	Aggressive	Anxious	Non	Non
	6A	85[a]	3.9	49.1	Non	Non	Non	Inhibited
	6B	18[a]	3.9	111.2	Aggressive	Anxious	Non	Inhibited
	6A	58[a]	4.9	80.7	Aggressive	Anxious	Inhibited	Inhibited
	6A	31[a]	3.9	66.5	Non	Non	Non	Non
	6A	34[a]	3.3	41.4	Aggressive	Non	Non	Non
	6A	35[a]	4.0	61.0	Non	Non	Non	Inhibited
	6B	46	2.8	33.9	Non	Non	Inhibited	Non
	6B	07	1.0	6.1	Non	Non	Non	Inhibited
	6B	26	1.7	7.7	Non	Non	Non	Non
	6B	39	1.3	11.1	Non	Non	Inhibited	Non
Male	7A	82[a]	3.1	72.7	Non	Non	Non	Inhibited
	7A	48[a]	3.0	75.0	Non	Non	Non	Inhibited
	7A	68[a]	3.3	95.2	Non	Non	Non	Non
	10	42[a]	4.2	167.3	Aggressive	Non	Non	–
	10	82[a]	3.1	46.1	Aggressive	Non	Non	–
	7B	57	1.4	48.2	Non	Anxious	Non	Inhibited
	7B	54	2.4	100.4	Non	Non	Inhibited	Inhibited
	7A	87	2.8	67.4	Non	Non	Inhibited	Inhibited
	7B	84	1.8	56.8	Non	Non	Non	Inhibited
	7B	40	2.1	60.4	Non	Non	Non	Non
	7B	25	2.3	68.7	Aggressive	Non	Non	Non
	7A	42	2.3	33.9	Non	Non	Non	Inhibited
	7A	90	2.0	32.7	Non	Non	Non	Non
	7A	11	1.8	35.9	Non	Non	Inhibited	Inhibited
	7A	16	1.9	20.5	Non	Non	Non	Non
	10	56	2.3	52.9	Non	Non	Inhibited	–
	10	02	2.3	41.3	Non	Non	Non	–
	10	41	2.4	60.6	Aggressive	Anxious	Non	–
	10	58	1.5	17.7	Non	Non	Non	–
	10	12	2.1	45.0	Non	Non	Non	–
	10	09	1.3	7.8	Non	Non	Non	–

[a] Indicates that the monkey was a heavy drinker, as defined by drinking >3.0 g/kg on average throughout 22-h access

Fig. 2a) and achieved significantly higher BECs (84 versus 46 mg%, $t_{30} = 3.1$, corrected $p = 0.012$, Fig. 2b) compared to non-aggressive monkeys. Water intake did not differ between aggressive and non-aggressive monkeys (211.5 versus 162.6 g/kg/day, $t_{30} = 1.6$, corrected $p = 0.38$, Fig. 2c). Baseline duration of extreme aggressive behavior positively correlated with future average daily ethanol intake ($R_s = 0.48$, $p = 0.005$) and future average BECs attained ($R_s = 0.38$, $p = 0.031$). All correlations are shown in Table 4. Finally, the relative risk of heavy drinking was significantly higher in aggressive versus non-aggressive monkeys, with aggressive monkeys at 200 % more at risk for becoming heavy drinkers than non-aggressive monkeys (Table 5).

No significant differences in ethanol intake, water intake, or BECs were observed between actively anxious and non-actively anxious monkeys or between behaviorally inhibited and non-behaviorally inhibited monkeys (Fig. 2). No significant correlations between durations of anxious behavior or latencies to interact with novel objects and ethanol or water intake-related variables were observed (Table 4). Active anxiety and behavioral inhibition did not significantly increase risk for heavy drinking (Table 5).

Sex differences in ethanol consumption and temperament

Female monkeys self-administered significantly more water on average (234.5 versus 143.5 g/kg/day, $t_{30} = 3.7$, corrected $p = 0.003$) than male monkeys. Ethanol self-administration and BECs attained by female and male monkeys did not significantly differ, though female ethanol intake was non-significantly higher (3.3 versus 2.4 g/kg/day, $t_{12.75} = 2.0$, corrected $p = 0.19$). Female monkeys reacted significantly more to the HIT stimuli, with significantly longer duration of open mouth threat during the stare phase of the HIT and freeze during the profile phase of the HIT (24 versus 5 % of the interval, $z = 2.9$, corrected $p = 0.023$; 94 versus 59 % of the interval, $z = 3.7$, corrected $p = 0.002$, respectively). Latencies to inspect, touch, and manipulate the novel objects during the NOT were similar in each sex, with the only trend being a shorter latency to touch Mr. Potato Head in the female

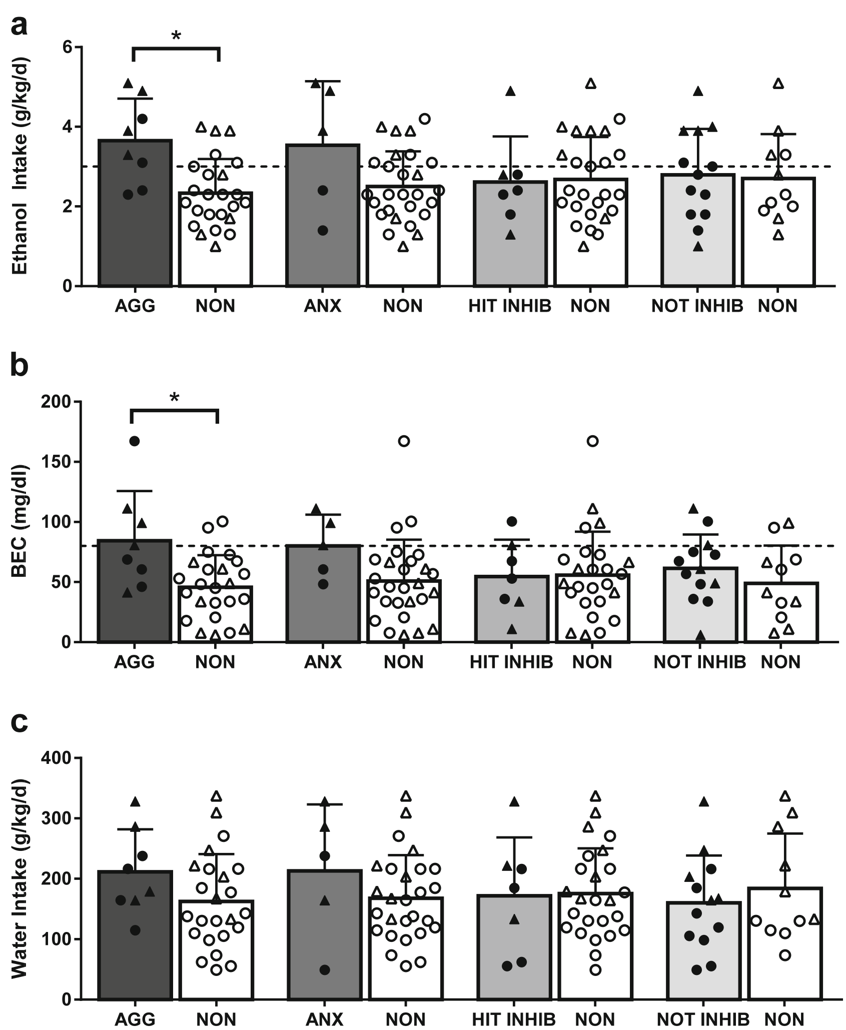

Fig. 2 Mean ± SD of **a** daily ethanol intake, **b** BEC, and **c** daily water intake over 12 months of 22-h ethanol access plotted by temperament group. Mean daily ethanol and water intakes (g/kg/day) calculated from an average of 351 days of self-administration/monkey/cohort (range 336–384 days). Average BEC (mg%) calculated from 63 samples/monkey/cohort (range 59–67 samples). Individual monkeys within each group depicted with *circles* (males) or *triangles* (females). *$p \leq 0.05$ (corrected), statistically significant group differences

Table 4 Spearman's rank-order correlations (R_s, p) describing the relationship between ethanol intake and intoxication during 22-h ethanol access and aggressive-like, anxious-like, and behaviorally inhibited behaviors during temperament testing

		Average daily intake (g/kg)	BEC (mg%)
HIT variables	Freeze profile	NS	NS
	Freeze stare	NS	NS
	Active anxiety	NS	NS
	Open mouth	NS	NS
	Extreme aggression	0.48, 0.005	0.38, 0.03
NOT variables	Inspect kiwi	NS	NS
	Touch kiwi	NS	NS
	Manipulate kiwi	NS	NS
	Inspect Mr. P	NS	NS
	Touch Mr. P	NS	NS
	Manipulate Mr. P	NS	NS
	Inspect snake	NS	NS
	Touch snake	NS	NS
	Manipulate snake	NS	NS

NS non-significant at $p > 0.05$

monkeys (195 versus 292 s, $z = 2.4$, corrected $p = 0.12$, data not shown).

Relationships between temperament variables

Behavioral inhibition was consistent across the NOT, with positive correlations observed between latencies to inspect, touch, and manipulate each object in both male and female monkeys (Supplemental Table 3). Monkeys with long latencies to interact with one novel object also displayed long latencies to interact with other novel objects, producing two distinct groups of anxious and non-anxious monkeys (data not shown). Latencies to interact with novel objects and durations of freezing during the HIT were not correlated (Supplemental Table 4).

Discussion

The results of this study demonstrate that measures of temperament in monkeys can be informative regarding future heavy ethanol consumption and intoxication. Specifically, baseline aggressive temperament and behavior were associated with increased risk of higher ethanol self-administration and intoxication when ethanol and water were concurrently available. Interestingly, despite baseline sex differences in behavioral reactivity to the HIT (with females demonstrating higher levels of reactivity [Fig. 3]), the association between temperament and ethanol drinking phenotypes was observed across male and female subjects. Conversely, in contrast to our hypotheses, anxiety-related behavior at baseline was not associated with future ethanol intake or intoxication and no significant differences in ethanol drinking were observed between anxious and non-anxious or behaviorally inhibited and non-behaviorally inhibited subjects.

The association between aggression and ethanol self-administration was also supported by correlations observed between aggressive behavior and ethanol self-administration and BECs. The duration of extreme aggression positively correlated with average daily intake of ethanol and BECs (Table 4). These correlations correspond with studies of adolescent children reporting that the magnitude of deviation in temperament from the mean of the general population is associated with the severity of drug use (Glanz and Pickens

Table 5 Drinking outcomes compared by baseline temperament

Rhesus monkey cohorts	Heavy drinkers ($n = 12$)	Non-heavy drinkers ($n = 20$)	Relative risk	
Characteristic	Percent	Percent	Value (95 % CI)	*p*
Aggressive ($n = 8$)	50	10	3.0 (1.3–6.7)	0.007
Active anxious ($n = 5$)	25	10	1.8 (0.7–4.4)	0.20
HIT inhibited ($n = 7$)	8.3	30	0.3 (0.05–2.1)	0.24
NOT inhibited ($n = 13$)[a]	60	50	1.3 (0.5–3.4)	0.63

[a] Only 24 subjects tested with the NOT (10 heavy drinkers)

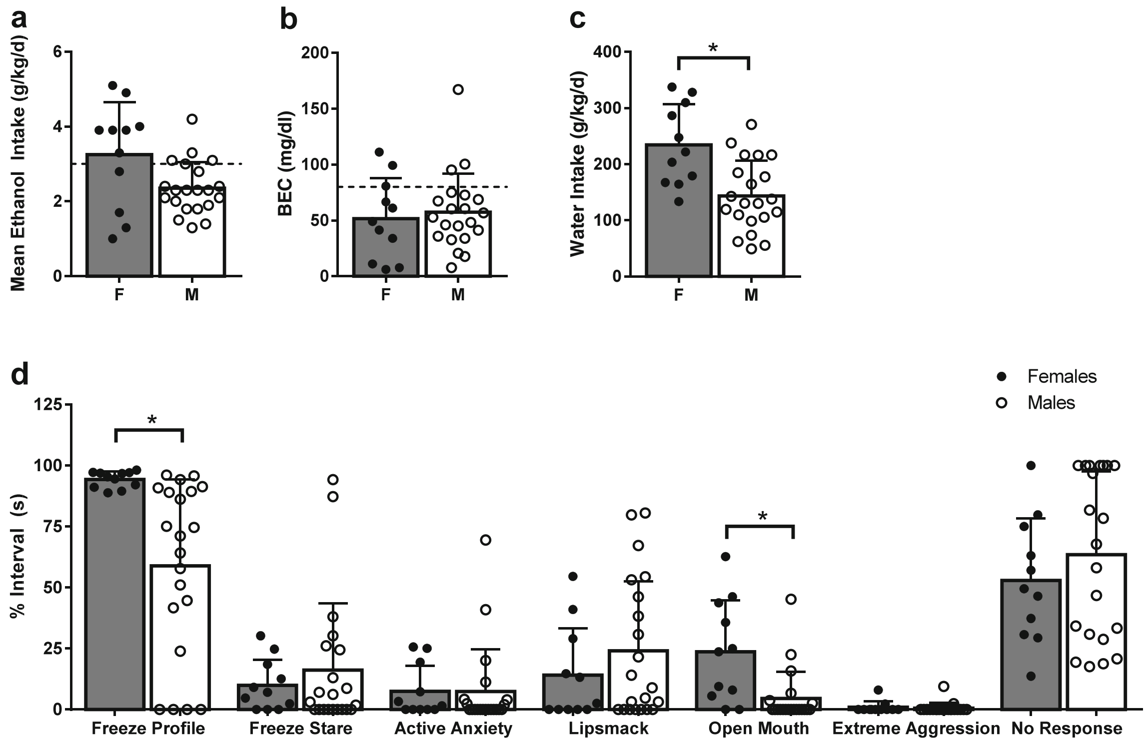

Fig. 3 Mean ± SD of **a** daily ethanol intake, **b** BEC, and **c** daily water intake over 12 months of 22-h ethanol access and mean ± SD of individual behavior durations during baseline HIT (**d**) plotted by sex. Mean daily ethanol and water intakes (g/kg/day) calculated from an average of 351 days of self-administration/monkey/cohort (range 336–384 days). Average BEC (mg%) calculated from 63 samples/monkey/cohort (range 59–67 samples). Individual monkeys within each group plotted with *circles*. *$p \leq 0.05$ (corrected), statistically significant group differences

1991; Tartar and Mezzich 1992). Overall, the association between aggression and alcohol self-administration is consistent with past research demonstrating an association between conduct disorder and substance abuse during adolescence, with childhood aggression and conduct disorder acting as predictors of future substance abuse (Brook et al. 1992; Boyle et al. 1999; Pardini et al. 2007; Fergusson et al. 2007; Jester et al. 2008). However, the literature on the relationship between alcohol and aggression in the human literature is largely non-specific regarding aggression subtypes. In this study, we specifically examined the predictive relationship between reactive aggression elicited by a social threat and future ethanol self-administration. Our results indicate a 200 % higher risk of aggressive subjects becoming heavy drinkers than non-aggressive subjects (Table 5), independent of sex, suggesting that further research should assess the role of reactive aggression versus controlled-instrumental aggression in drinking in human subjects. Additionally, some studies with human subjects have focused specifically on the relationship between alcohol and aggression in male subjects (Dolan et al. 1993; Ensminger et al. 1983; Kellam et al. 1975). Our results suggest that aggressive behavior may act as an important risk factor in both male and female subjects and that more emphasis should be placed on assessing aggressive behavior in both male and female subjects.

Although alcohol self-administration did not significantly differ between actively anxious and non-actively (3.6 versus 2.5 g/kg/day, $t_{30} = 2.1$, respectively; corrected $p = 0.13$), the actively anxious group was on average above the 3.0 g/kg/day used to demarcate heavy versus non-heavy drinking in this model. This suggests that more than 32 rhesus monkeys may be needed to document a significant effect of anxious behavior on attaining future status as a heavy alcohol drinker in this model. Importantly, only five subjects were classified as actively anxious, again suggesting that a large sample of monkeys may be needed to observe a predictive relationship between anxious behavior and future alcohol intake. Interestingly, there was an association between active anxiety and extreme aggression (data not shown, $R_s = 0.60$, $p = 0.0003$, $n = 32$) consistent with studies that have found a relationship between anxiety and externalizing disorders in youth (Marmorstein 2007).

Together, these data suggest that external expressions of anxiety and aggression rather than inhibition in response to stressful stimuli may be related to heavier ethanol intake. This relationship is also suggested by the association between the personality construct of behavioral undercontrol and substance use in prior research. Behavioral undercontrol includes characteristics such as negative emotionality, low constraint, risk-taking, and sensation-seeking and is also component of externalizing childhood psychiatric disorders such as conduct disorder. Our data suggests that actively anxious behaviors such as teeth grinding and yawning, sometimes described as displacement behaviors (Maestripieri et al. 1992; Schino et al. 1996), may relate to aggressive defensive behaviors elicited by the HIT and to a lesser degree future ethanol self-administration. Although past non-human primate studies have suggested a relationship between anxiety and alcohol intake, with macaque monkeys treated with the anxiolytic drug buspirone decreasing alcohol preference (Collins and Myers 1987), our results indicate that ethanol intake may only relate to specific components of anxiety, particularly those related to behavioral undercontrol rather than behavioral inhibition.

Most frequently, the HIT and NOT tests are to assess behavioral inhibition and temperament in infant subjects, which differs from the late adolescent population tested in this study. Our results indicate that the HIT can also be used as an assessment tool in late adolescent-young adult subjects, although the behaviors observed may differ in type and frequency when compared to infant subjects. For example, infant subjects frequently exhibit distress vocalizations such as coos which were not observed in our subjects (Gottlieb and Capitanio 2013). Importantly, it appears that adult subjects show similar responses to the HIT and seem to use similar behavioral strategies in response to threat as infants. Kalin and Shelton initially proposed two strategies: behavioral inhibition and aggression (1989), whereas more recent research has suggested additional factors such activity and emotionality (Kinnally et al. 2010) and anxiety (Gottlieb and Capitanio 2013). Our behavioral data suggest similar predominant strategies, with our variable analyses finding similar relationships among variables and allowing multiple variables within a single category to be collapsed (see Materials and methods section and Supplementary Figures). Interestingly, sex differences in response to these tests were also observed, particularly during the HIT.

However, when compared by sex, ethanol self-administration was comparable in male and female monkeys. Sex differences in ethanol self-administration using macaque monkeys are not always found, with reports of similar levels of self-administration between male and female cynomolgus (Pakarinen et al. 1999) and rhesus monkeys (Vivian et al. 1999) or higher levels of ethanol self-administration in male cynomolgus (Vivian et al. 2001; Grant et al. 2008b) and rhesus monkeys (Fahlke et al. 2000). However, a direct comparison between these studies is very difficult as rearing and housing conditions, age, and ethanol concentration and access differ. The relationship of these temperament measures to stress and gonadal hormones may provide useful information regarding the role of sex in behavioral responses and subsequently help to explain sex effects on ethanol self-administration.

In conclusion, late adolescent rhesus monkeys demonstrate individual differences in ethanol self-administration and behavioral responses during temperament testing. Specifically, the degree of aggression observed during testing was associated with future ethanol consumption and intoxication. The differences observed are analogous to the differences observed in human adolescents, with aggression serving as a potent predictor of future substance abuse (Disney et al. 1999; Boyle et al. 1999; Fothergill and Ensminger 2005, Pardini et al. 2007; Fergusson et al. 2007; Jester et al. 2008). The potential relationship between anxiety, aggression, and ethanol self-administration should be further examined in the context of the associations between the neuroendocrine response, anxious and aggressive temperaments, and alcohol consumption. The inclusion of post-ethanol exposure temperament tests in future groups undergoing ethanol self-administration will allow for the analysis of changes in anxious and aggressive behavior due to chronic ethanol consumption.

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