Risk preference following adolescent alcohol use is associated with corrupted encoding of costs but not rewards by mesolimbic dopamine

Nicholas A. Nasrallah[a,1], Jeremy J. Clark[b,c,1,2], Annie L. Collins[a], Christina A. Akers[b,c], Paul E. Phillips[b,c,d], and Ilene L. Bernstein[a,d]

Departments of [a]Psychology, [b]Psychiatry and Behavioral Sciences, and [c]Pharmacology, [d]Graduate Program in Neurobiology and Behavior, University of Washington, Seattle, WA 98195

Edited by Ranulfo Romo, Universidad Nacional Autonoma de Mexico, Mexico D.F., Mexico, and approved February 17, 2011 (received for review November 26, 2010)

Several emerging theories of addiction have described how abused substances exploit vulnerabilities in decision-making processes. These vulnerabilities have been proposed to result from pharmacologically corrupted neural mechanisms of normal brain valuation systems. High alcohol intake in rats during adolescence has been shown to increase risk preference, leading to suboptimal performance on a decision-making task when tested in adulthood. Understanding how alcohol use corrupts decision making in this way has significant clinical implications. However, the underlying mechanism by which alcohol use increases risk preference remains unclear. To address this central issue, we assessed dopamine neurotransmission with fast-scan cyclic voltammetry during reward valuation and risk-based decision making in rats with and without a history of adolescent alcohol intake. We specifically targeted the mesolimbic dopamine system, the site of action for virtually all abused substances. This system, which continuously develops during the adolescent period, is central to both reward processing and risk-based decision making. We report that a history of adolescent alcohol use alters dopamine signaling to risk but not to reward. Thus, a corruption of cost encoding suggests that adolescent alcohol use leads to long-term changes in decision making by altering the valuation of risk.

Alcohol was recently shown to be among the most harmful drugs of abuse to individuals and society overall (1). During adolescence, a critical period of neurobiological development, individuals often receive their first exposure to alcohol, and a significant proportion do so during episodes of high intake or bingeing (2, 3). Such experience can be antecedent to problem drinking (3) and is associated with impairments in decision making (4). Recently, it has been demonstrated, via an alcohol "Jello shot" protocol, that high levels of voluntary alcohol intake during adolescence produces long-term perturbations of risk-based decision making in rodents (5), leading to a suboptimal preference for risk. Risky decisions are those where a large but uncertain reward is favored over a smaller certain reward, a process thought to be mediated by the ventral striatum (6, 7). Like virtually all drugs of abuse, alcohol alters dopamine transmission within the ventral striatum, which is a primary target of midbrain dopamine neurons (8, 9). Phasic increases in dopamine transmission are evoked by rewarding outcomes and associated cues (10, 11), both of which have been shown to scale with the magnitude and the probability of reward (12, 13). Indeed, it has been suggested that midbrain dopamine neurons specifically encode risk along with reward value (14), and models of probabilistic choice have implicated the ventral striatum in mediating risk-associated decisions (6, 7, 15–16). Similarly, phasic dopamine signaling within the ventral striatum has been previously linked to value-based decision making (13, 17). Thus, encoding of value and risk in the cost–benefit computations necessary for adaptive decision making (14, 18) may be particularly vulnerable to disruption by drugs that target the dopamine system.

Voluntary ingestion of alcohol by adolescent rats significantly impairs their performance on a probability-discounting task when they are adults (5). This impairment results in a suboptimal preference for risky options. The expected value of an option is equal to the value of the potential reward discounted by the costs associated with that option. Thus, the maladaptive bias toward large but risky outcomes displayed by these rats suggests that they overvalue larger rewards and/or fail to appropriately discount that value based on its diminished probability of occurrence. Indeed, maladaptive reward valuation and maladaptive cost discounting are two primary vulnerabilities in the decision-making apparatus proposed to be exploited by abused substances (19). The neural circuits implicated in the processing of these vital components of decision making, including dopamine and cortical systems, continue to develop throughout the adolescent period (3, 20). Previous work assessing the consequences of drug use on future reward processing has shown that a history of drug exposure may alter neural and behavioral responses to natural rewards, effects thought to be mediated by dopamine systems (21). A corruption of reward valuation could promote maladaptive and suboptimal behavior by placing excessive priority on seeking rewards such as food, drugs, or sex. Accordingly, one potential explanation of risk-biased choice behavior in rats with a history of adolescent alcohol use is that sensitization of dopamine systems alters the processing of rewards (22). Another potential, although not mutually exclusive, explanation is that a history of drug exposure may alter the influence of the probability of reward occurrence on choice behavior. That is, drug use may corrupt cost encoding and thereby alter decisions that rely on this altered valuation of cost. A specific effect on cost encoding may promote maladaptive behavior by diminishing the ability to accurately assess the risk associated with the outcomes of various behaviors (e.g., gambling or drug use). Thus, risk preference may result from maladaptive cost discounting wherein drug exposure reduces the discounting of rewards based on the probability costs associated with procuring them (19, 23). The studies reported here tested these two explanations of the alcohol-induced increase in risk preference by using in vivo fast-scan cyclic voltammetry (FSCV) to assess phasic dopamine signaling to rewards and to cues predicting risky or certain outcomes. We report that risk preference after adolescent alcohol use is associated with corrupted encoding of costs but not rewards.

Author contributions: N.A.N., J.J.C., and I.L.B. designed research; N.A.N., J.J.C., A.L.C., and C.A.A. performed research; N.A.N. and J.J.C. analyzed data; P.E.P. contributed new reagents/analytic tools; and N.A.N., J.J.C., P.E.P., and I.L.B. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]N.A.N. and J.J.C. contributed equally to this work.

[2]To whom correspondence should be addressed. E-mail: jjc1@uw.edu.

Results

Alcohol Intake and Risk Preference. Adolescent rats [postnatal day (PND) 30–49] were provided with continuous access to gelatin shots (10% EtOH or control gelatin) for 20 d (Fig. 1*A*). Daily adolescent alcohol intake averaged 10.4 g/kg with a range of 6.0–15.5 g/kg. There were no significant differences in body weight between alcohol-exposed (234.2 ± 8.6 g) and control (245.0 ± 11.6 g) animals after the 20 d of gelatin access [$t(8) = 0.748$, $P = 0.476$].

Three months after cessation of alcohol access, all animals were tested on a probability-discounting task to assess risk preference. Analysis of choice behavior generated probability-discounting curves with decreasing probability of large reward leading to increases in choice of the small, certain reward option ($F_{2, 16} = 8.454$, $P = 0.003$; Fig. 1*B*). Adolescent alcohol exposure increased preference for large, probabilistic rewards over small, certain rewards during choice trials ($F_{2, 16} = 7.684$, $P = 0.024$; Fig. 1*B*). Notably, at the 50% probability condition, where the two options have equal expected values but the probabilistic option has maximal uncertainty, alcohol-exposed rats displayed greater preference for the large, probabilistic reward [$t(8) = 3.149$, $P = 0.014$, Fig. 1*B*].

Phasic Dopamine Signaling to Risk. Experiment 1 assessed risk valuation after a history of alcohol use by analyzing dopamine signaling to cues (lever presentations) indicating risky (DA_R) or certain outcomes (DA_C) during forced trials of the decision-making task (Fig. 1 *C–E*). Dopamine signals were used to create within-subjects ratio scores (DA_R/DA_C) to compare risk valuation between groups. Alcohol-exposed animals had a significantly greater ratio score across probabilities compared with controls ($F_{1, 6} = 14.95$, $P = 0.008$), indicating significantly greater dopamine signaling to risk in these animals (Fig. 1*C*). At 50% probability, DA_R was not different from DA_C in controls [$t(3) = 1.020$, $P = 0.383$; Fig. 2*A*] but significantly diverged in alcohol-exposed rats [$t(3) = 3.340$, $P = 0.044$; Fig. 2*B*], yielding a greater ratio score for alcohol-exposed animals [$t(6) = 4.052$, $P = 0.007$; Fig. 1*C*].

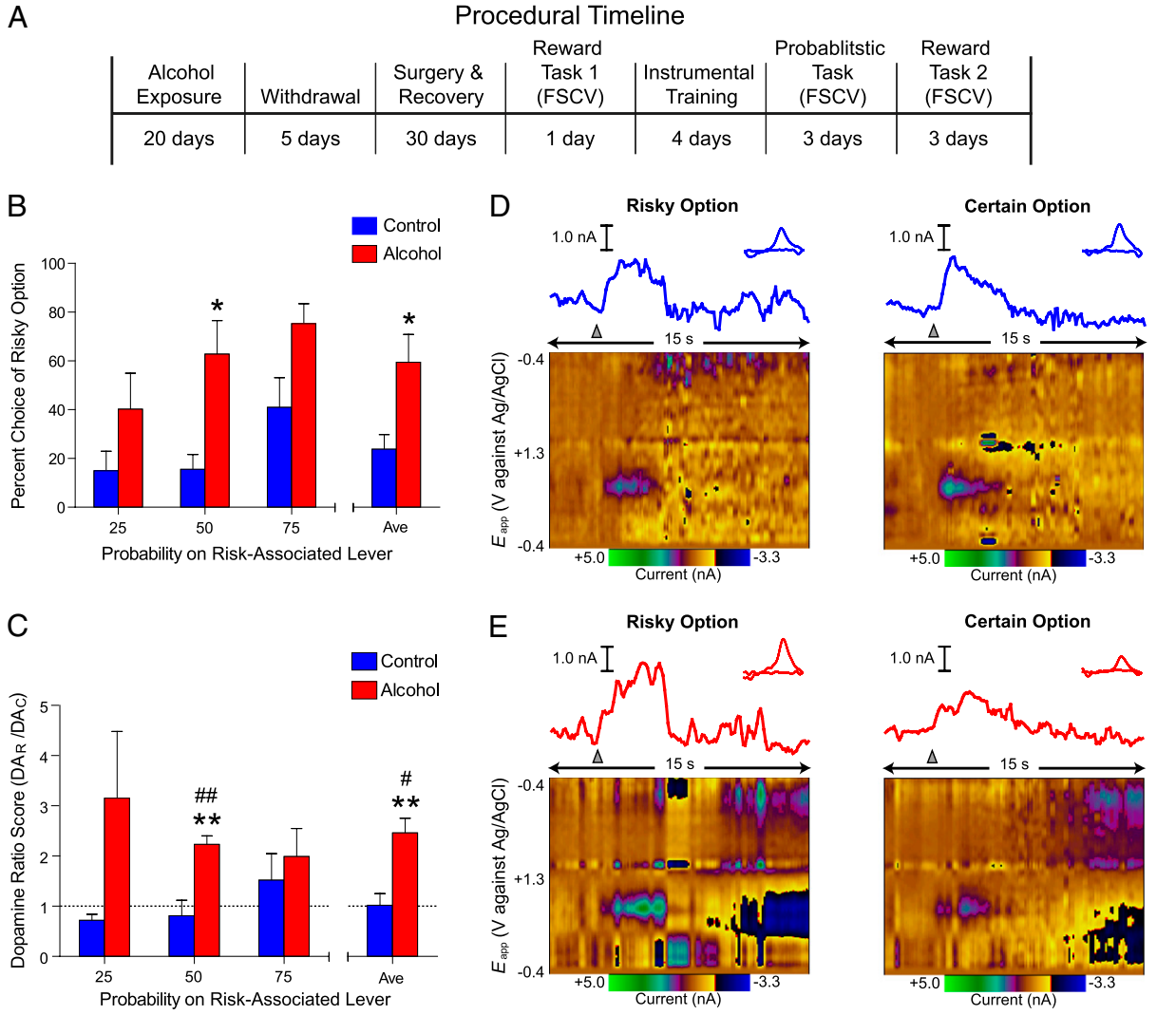

Fig. 1. Behavior and phasic dopamine signaling during probability discounting. (*A*) Timeline of experimental procedures. (*B*) Probability discounting across conditions. (*C*) Dopamine ratio scores (DA_R/DA_C) across conditions. (*D* and *E*) Representative traces (*Upper*) and corresponding background-subtracted cyclic voltammograms (*Lower*) depict changes in dopamine oxidative current within the AcbC in response to presentation (arrowhead) of levers predicting risky and certain outcomes at 50% probability in control (*D*) and alcohol-treated (*E*) animals. The pseudocolor plots depict color-coded observed changes in redox currents as a function of applied potential (*y* axis) plotted over time (*x* axis). Data are represented as means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ (between groups); $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ (compared with 1).

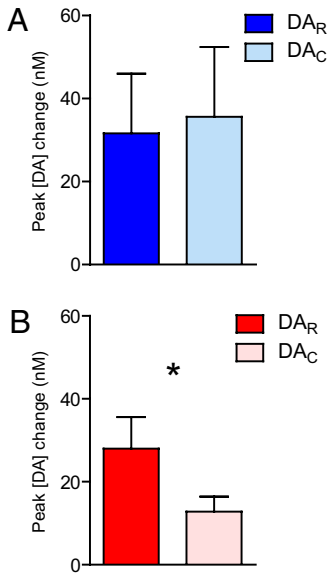

Fig. 2. Phasic dopamine signaling in response to the presentation of risky and certain options during the 50% probability condition for alcohol-exposed (red; *B*) and control (blue; *A*) animals. At 50% probability, DA_R was similar to DA_C in controls [$t(3) = 1.020$, $P = 0.383$] but diverged in alcohol-exposed rats [$t(3) = 3.340$, $P = 0.044$]. These peak amplitude responses were used to create within-subjects ratio scores (DA_R/DA_C) to compare risk valuation between groups. Data are presented as means ± SEM peak change in dopamine concentration. *$P > 0.05$.

The expected values for both the risky and certain options are equivalent in the 50% condition and when averaged across all conditions ($E_V = 2$ pellets); thus, DA_R and DA_C signals would be expected to yield ratio scores approximating 1. For either of these conditions, a score significantly greater than 1 would indicate greater dopamine signaling to risk. Alcohol-exposed rats demonstrated ratio scores significantly greater than 1 at 50% [$t(3) = 7.17$, $P = 0.006$] and averaged across conditions [$t(3) = 4.952$, $P = 0.016$; Fig. 1*C*], whereas the ratio score for controls did not differ from 1 [50%: $t(3) = 0.616$, $P = 0.581$; Average: $t(3) = 0.84$, $P = 0.939$].

Phasic Dopamine Signaling to Reward. Experiment 2 assessed reward valuation after a history of alcohol use by randomly delivering sucrose rewards of varying magnitudes (1, 2, and 4 pellets) and recording dopamine signaling in response to these rewards in two separate sessions—before and after the decision-making task. Phasic dopamine release within the nucleus accumbens core (AcbC) increased with reward magnitude (before: $F_{2, 12} = 5.274$, $P = 0.023$; after: $F_{2, 12} = 17.32$, $P = 0.0003$). However, no differences in dopamine signaling to rewards, at any value, were observed between alcohol-exposed and non-exposed rats (before: $F_{1, 6} = 0.282$, $P = 0.614$; after: $F_{1, 6} = 2.386$, $P = 0.733$) (Fig. 3). The pattern of signaling was consistent when phasic dopamine transmission to reward was assessed before or after the probability-discounting task. Mixed-measures ANOVA revealed a main effect of reward value ($F_{2, 12} = 5.274$, $P = 0.023$), but no main effect of alcohol treatment ($F_{1, 6} = 0.282$, $P = 0.614$) or interaction ($F_{2, 12} = 0.678$, $P = 0.526$), before the probability-discounting task and a main effect of reward value ($F_{2, 12} = 17.32$, $P = 0.0003$), but no main effect of alcohol treatment ($F_{1, 6} = 2.386$, $P = 0.733$) or interaction ($F_{2, 12} = 1.465$, $P = 0.296$), after the probability-discounting task.

Discussion

Here, we assessed two separate neuroeconomic explanations for alcohol's long-term effects on decision making: altered cost valuation and altered reward valuation. Alterations in cost discounting—the ability to accurately devalue outcomes because of increases in associated costs—could explain a shift in risk preference by underestimating the risk associated with probabilistic options. Previous studies have shown that a history of chronic drug exposure alters discounting of delayed rewards, resulting in impulsivity (23). Similar maladaptive discounting processes resulting from drug exposure may affect choices associated with other costs, such as risk (15). Indeed, the results of Experiment 1 demonstrate that risk preference after a history of adolescent alcohol exposure is associated with altered dopamine signaling to risk.

Maladaptive choice behavior may also result from altered reward valuation, where a perturbation of a biological reward signal—proposed to be mediated by midbrain dopamine—would reflect an aberrant value and/or quality of an outcome (24). Neural changes in the value-signaling component of the decision-making system, such as overvaluation of expected outcomes, could result from alcohol exposure's direct effect on reward systems. This theory relies on evidence demonstrating that drugs of abuse usurp and persistently alter brain systems that mediate natural reward (25). In Experiment 2, however, no evidence was found to support this hypothesis. Although it remains possible that other approaches could yield evidence of altered reward valuation, the current work demonstrates that risk preference after adolescent alcohol use is associated with corrupted encoding of risk but not rewards by midbrain dopamine.

It remains to be determined whether the altered risk processing detected in Experiment 1 reflects added value conferred by risk (12, 14) (i.e., a gambling buzz) or diminished subjective probability assessment (i.e., an incomplete estimation of the decreasing probability). Probability estimation likely involves cortical brain regions, including the orbitofrontal cortex (26), an area still developing during the adolescent period (20). A neurobiological perturbation of cortical development by alcohol may promote aberrant risk encoding where the assignment of excessive value to a risky option by phasic dopamine release in the striatum biases choice behavior. A change in the utility of the risky option could also result from an increased sensitivity to outcomes that are better than expected after probabilistic predictors and/or decreased sensitivity to outcomes that are worse than expected. Indeed, an imbalance in sensitivity to positive and negative outcomes has been previously implicated in risk-seeking behavior (27). Future work examining these hypotheses may further clarify the neuroeconomic consequences of adolescent alcohol exposure.

The neurobiological systems that are corrupted by voluntary alcohol consumption during adolescence and by what mechanism alcohol produces such effects remain open questions. The findings reported here suggest that a history of alcohol does not lead to a general perturbation of dopamine systems, as indicated by a lack of effect on dopamine signaling to deterministic rewards. Rather, the effect on dopamine signaling shown here is limited to probabilistic conditions where cortical regions may play a more significant role.

These studies demonstrate that increased risk preference after adolescent alcohol use is associated with elevated phasic dopamine transmission in response to risky options, but not in response to rewards alone. This disruption is not associated with a general corruption of the reward system; rather, it is specific to the cost valuation essential to decision making. Therefore, we conclude that enduring perturbation of decision-making processes, reflected in phasic dopamine transmission, may result

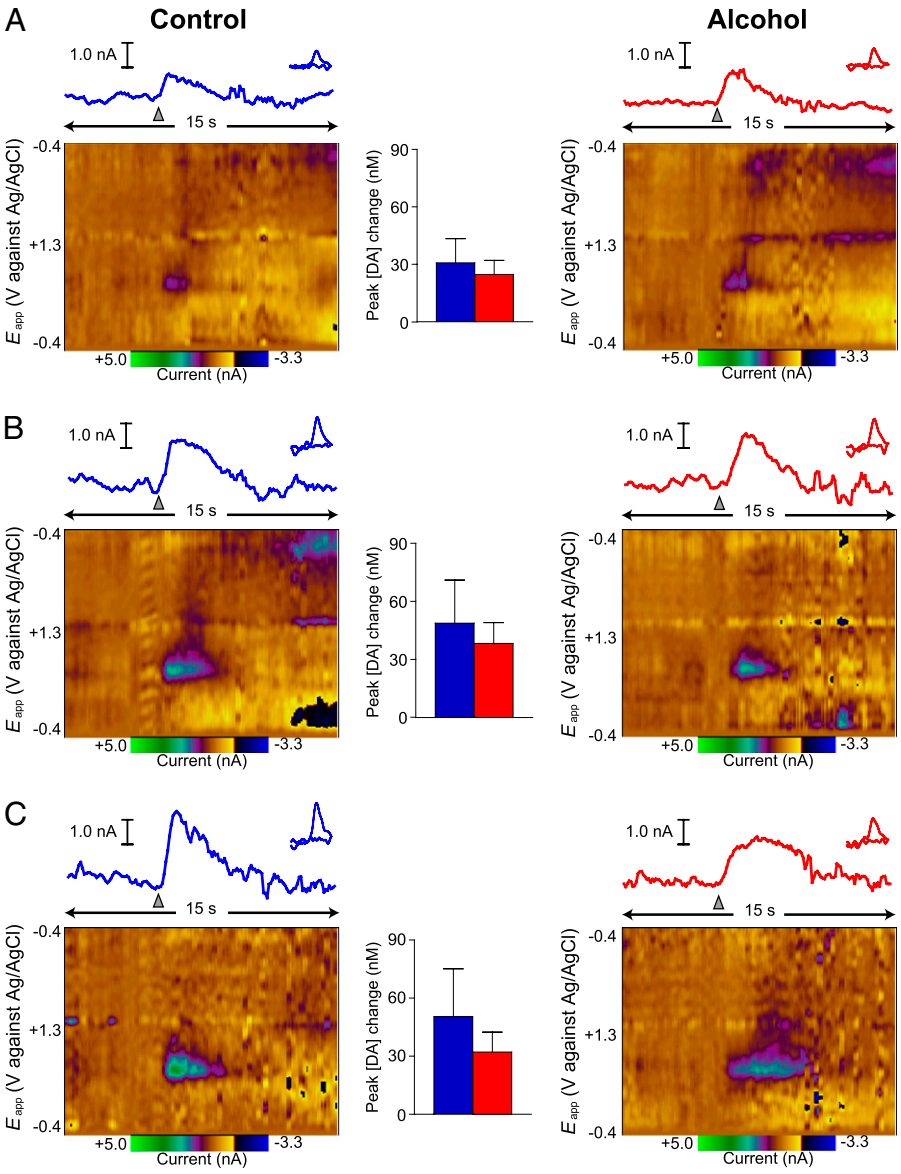

Fig. 3. Phasic dopamine signaling during the free reward task. Representative traces (*Upper*) and corresponding background-subtracted cyclic voltammograms (*Lower*) depict changes in dopamine oxidative current within AcbC to delivery (arrowhead) of one (*A*), two (*B*), or four (*C*) sucrose pellets for alcohol-exposed (red) and control (blue) animals. The pseudocolor plots depict color-coded observed changes in redox currents as a function of applied potential (*y* axis) plotted over time (*x* axis). Bar graphs (*Center*) represent means ± SEM peak dopamine concentration for each value. No differences were observed between groups at any value: (*A*) $t(6) = 0.422$, $P = 0.688$; (*B*) $t(6) = 0.422$, $P = 0.688$; (*C*) $t(6) = 0.681$, $P = 0.522$.

from the modulation of risk valuation independent of reward valuation. This work significantly expands our understanding of how decision making is corrupted by drug exposure and provides a preclinical model for characterizing changes in brain valuation that underlie alcohol's effect on risky decision making.

Materials and Methods

General Methodology. We provided adolescent rats (PND 30) with continuous access to an alcohol (10%) or control gelatin for 20 d (5). After cessation of alcohol access, we implanted rats with carbon-fiber microelectrodes in the AcbC for detection of dopamine transmission by in vivo FSCV (28). After recovery from surgery, we food-restricted rats to ~90% free-feeding weight and trained them to press a lever for food in a standard operant chamber. We then used FSCV to measure dopamine transmission during adulthood (PND 140). In Experiment 1, we trained and tested rats on a probability-discounting task, offering a choice between a small but certain option (two pellets at 100% reinforcement rate) or a large but risky option (four pellets at 75%, 50%, and 25% reinforcement rate) contingent on lever responding. Lever presentations served as predictive signals for probabilistic or certain outcomes and were presented separately during forced trials and simultaneously during choice trials. We assessed preference behaviorally during choice trials and phasic dopamine responses to predictive cues during forced trials (13). In Experiment 2, we delivered unpredicted rewards of varying magnitude (one, two, and four pellets) during a single testing session before operant training for Experiment 1 and compared phasic dopamine signaling to reward delivery between groups. Phasic dopamine signaling to unpredicted rewards was tested again after the probability-discounting task. All procedures were conducted in accordance with University of Washington Institutional Animal Care and Use Committee guidelines.

Animals and Housing. Ten male Sprague–Dawley rats aged PND 27 at the start of the experiments were used in the studies. Adolescent alcohol exposure took place from PND 30–49. Animals were housed individually in polycarbonate tubs on a 1-h light/dark schedule. Teklad rodent chow and water was available ad libitum except as noted. Animals were weighed and handled for several minutes daily throughout the course of all experiments.

Alcohol Preparation/Administration. Alcohol was presented to rats in a gel composed of distilled water, Knox gelatin, Polycose (10%), and EtOH (10%). This gelatin was made available 24 h/d for 20 d in addition to standard chow and water. Preparation followed the methodology of Rowland et al. (29). Briefly, water was boiled, and gelatin powder (3 g/100 mL; Knox) and Polycose (10% by weight) were added. For alcohol gelatin, ethanol (10% by volume) was added to the solution, and the mixture was poured into individual glass jars (~60 mL). Jars were sealed and refrigerated overnight. This procedure was designed to minimize evaporation of ethanol and has been validated to yield accurate ethanol content (29) and to lead to alterations in brain chemistry (30, 31). Alcohol presentation involved allowing jars of gelatin to warm to room temperature and recording their weight. Jars were then placed into an animal's cage, replacing the old jar every 24 h. During this time, rats were weighed and handled. Finally, weights of the jars from the previous day were recorded, and consumption of alcohol (in g/kg) was calculated for each animal. Experiments began with 3 d of preexposure to a control gelatin, and all animals were matched by weight and baseline intake and split into two conditions—one group received 24-h access to an alcohol gelatin and the other received a control gelatin for 20 d. Daily alcohol intake averaged 10.4 g/kg with a range of 6.0–15.5 g/kg. There were no significant differences in body weight between groups after 20 d [$t(8) = 0.748$, $P = 0.476$].

Behavioral Monitoring and Alcohol Withdrawal. After 20 d of alcohol exposure, animals were withdrawn from ethanol. Animals were monitored daily for changes in weight and behavior during ethanol exposure and withdrawal. During daily handling, we looked for abnormalities such as excessive locomotor activation, muscle rigidity, clonus, tremors, or convulsions. At no time during the studies did we observe sustained tremors or vulnerability to audiogenic seizures. Unlike more commonly used ethanol self-administration procedures, which require food or fluid deprivation, this method is associated with considerable voluntary intake without the need for deprivation. No adverse effects on the health of the animals have been seen.

Voltammetry Surgery. Surgical preparation for in vivo voltammetry used aseptic technique. Rats were anesthetized with isoflurane and placed in a stereotaxic frame. The scalp was swabbed with 10% povidone iodine, bathed with a mixture of lidocaine (0.5 mg/kg) and bupivacaine (0.5 mg/kg), and incised to expose the cranium. Holes were drilled and cleared of dura mater above the AcbC (1.3 mm lateral and 1.3 mm rostral from bregma) and at convenient locations for a reference electrode and three anchor screws. The reference electrode and anchor screws were positioned and secured with cranioplastic cement, leaving the working electrode holes exposed. Once the cement cured, the microsensors were attached to the voltammetric amplifier and lowered into the target recording regions (AcbC; 6.8 mm ventral of dura mater). Finally, cranioplastic cement was applied to the part of the cranium still exposed to secure the working electrode.

Electrochemical Detection of Dopamine. During all experimental sessions, chronically implanted microsensors were connected to a head-mounted voltammetric amplifier for dopamine detection by FSCV (28). Voltammetric scans were repeated every 100 ms to obtain a sampling rate of 10 Hz. When dopamine is present at the surface of the electrode during a voltammetric scan, it is oxidized during the anodic sweep to form dopamine-*o*-quinone (peak reaction at approximately +0.7 V), which is reduced back to dopamine in the cathodic sweep (peak reaction at approximately −0.3 V). The ensuing flux of electrons is measured as current and is directly proportional to the number of molecules that undergo the electrolysis. The redox current obtained from each scan provides a chemical signature that is characteristic of the analyte (cyclic voltammogram), allowing resolution of dopamine from other substances. For quantification of changes in dopamine concentration over time, the current at its peak oxidation potential can be plotted for successive voltammetric scans. Waveform generation, data acquisition, and analysis were carried out on a PC-based system using two PCI multifunction data acquisition cards and software written in LabVIEW (National Instruments). Voltammetric data analysis was carried out by using software written in LabVIEW (National Instruments) and low-pass filtered at 2,000 Hz. Dopamine was isolated from the voltammetric signal with chemometric analysis (32) using a standard training set based on stimulated dopamine release detected by chronically implanted electrodes. Dopamine concentration was estimated based on the average postimplantation sensitivity of electrodes (28).

Testing and Recording Procedures. All behavioral testing and voltammetry recordings took place in operant chambers (Med Associates). Before free-pellet testing, rats were food-restricted to maintain them at ~90% of their free-feeding weight. At 2 d before testing, 45 mg of sucrose pellets (Bio Serve) were offered in the home cage to minimize neophobia. Before the beginning of all experimental sessions (free-pellet task and probability-discounting task), microelectrodes were connected to a head-mounted voltammetric amplifier for dopamine detection by FSCV (described above). Head-mounted amplifiers interfaced with a PC-driven data acquisition system through an electrical swivel mounted above the operant chamber.

Reward Magnitude Task 1. A single test session was conducted where rewards were delivered at varying magnitudes (one, two, and four pellets) on a variable-interval 90-s schedule. The session consisted of five trials at each reward magnitude with a randomized reward sequence. These sessions allowed for recording of phasic dopamine to each reward magnitude, and signals were averaged for each value across the session. Peak dopamine in response to reward delivery was obtained by taking the largest value in the 2-s period after stimulus presentation. Peak values for the free-pellet task were then compared by using mixed-measures ANOVA with pellet value as the repeated measure and alcohol treatment as the between-group measure. All post hoc comparisons were made with the Bonferroni correction for multiple tests. All statistical analyses were carried out with Prism (GraphPad Software).

Probability-Discounting Task. Animals were first magazine trained and then trained on a fixed-ratio, one discrete-operant schedule for 45-mg sucrose pellets (Bio Serve) on both levers to a criterion of 80% response rate in a session (30 trials per session). Animals were then tested on a concurrent instrumental task involving the presentation of two levers, one associated with the certain delivery (100%) of two sucrose pellets and the other associated with the probabilistic delivery (either 75%, 50%, or 25%) of four pellets. Each daily 45-min session consisted of 24 forced trials followed by 24 free-choice trials. At the start of each session, the chamber was in the intertrial interval state—completely dark with no light cues. All trials began with illumination of the house light and a light in the food tray cueing the animal to make a nose poke into the food tray within 10 s, which ensured that the subject was centered in the chamber at the start of each trial, eliminating position bias. Failure to nose poke resulted in trial termination, and the chamber returned to the intertrial interval state. During training, animals were exposed to forced trials wherein a successful nose poke led to the extension of a single lever, presented pseudorandomly. A response was required within 10 s, or the trial was terminated and the chamber returned to the intertrial interval. A successful response resulted in the illumination of the tray light and delivery of reward, based on the associated probability, followed by an intertrial interval of 45 s. Forced-trial sessions consisted of 24 trials, which served to expose the animal to each option and its associated expected value.

During each session, forced-choice trials were followed by free-choice trials with the same probability for the uncertain lever. Free-choice trials followed the guidelines described above, but each successful nose poke resulted in the extension of both levers, and the animal was free to choose between the two levers within 10 s. Thus, this session offered the animal a choice between the two levers to assess the animal's preference between options. Lever choice was recorded and analyzed by using repeated-measures ANOVA, with

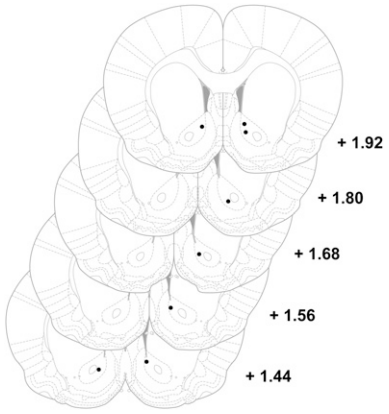

Fig. 4. Histological verification of recording sites. The number for each plate indicates millimeters from bregma (33).

probability as repeated measure and alcohol treatment as the between-group measure, and post hoc *t* test. With one lever/light as the cue for certain delivery of two pellets (12 trials) and the other as the cue for uncertain delivery of four pellets (12 trials), we assessed phasic dopamine release in response to the presentation of these cues during forced trials across the range of probabilities (i.e., 75%, 50%, and 25%). These signals were used to create a within-subjects ratio score (DA_R/DA_C) to compare risk valuation across groups. Because dopamine transmission during the initial forced trials of a new session likely reflects the previously learned probability (or nothing for the first probability condition), we restricted our analysis to the last 12 forced trials (6 trials for DA_R and 6 trials for DA_C) for each session. Ratio scores were analyzed by using mixed-measures ANOVA, with probability as repeated measure and alcohol treatment as the between-group measure, and post hoc *t* test. Comparison of each group's averaged and 50% ratio score to 1 was made with one-sample *t* tests.

Reward Magnitude Task 2. After the probability-discounting task was concluded, three additional test sessions were conducted where rewards were delivered at varying magnitudes (one, two, and four pellets) on a variable-interval 90-s schedule. Each session consisted of 20 trials in which a single reward magnitude was delivered, counterbalanced within each group for order of magnitude exposure. These sessions allowed for multiple recordings of phasic dopamine in response to each reward magnitude. Peak dopamine in response to reward delivery was obtained by taking the largest value in the 2-s period after stimulus presentation. Peak values for the free-pellet task were then compared by using mixed-measures ANOVA, with pellet value as the repeated measure and alcohol treatment as the between-group measure. All post hoc comparisons were made with the Bonferroni correction for multiple tests. All statistical analyses were carried out with Prism (GraphPad Software). The results of this experiment revealed a main effect of reward value ($F_{2,\ 12}$ = 17.32, P = 0.0003), but no main effect of alcohol treatment ($F_{1,\ 6}$ = 2.386, P = 1.733) or interaction ($F_{2,\ 12}$ = 1.465, P = 0.296), after the probability-discounting task.

Histological Verification of Recording Sites. On completion of the experiments, recording sites were localized by using standard histological procedures. Animals were anesthetized with sodium pentobarbital, and the recording site was marked with an electrolytic lesion (300 V) by applying current directly through the recording electrode for 20 s. Animals were transcardially perfused with PBS followed by 4% paraformaldehyde. Brains were removed and postfixed in paraformaldehyde for 24 h and then rapidly frozen in an isopentane bath (~5 min), sliced on a cryostat (50-μm coronal sections, 20 °C), and stained with cresyl violet to aid in visualization of anatomical structures. Recording sites were predominately identified in the medial AcbC (Fig. 4). Two animals (one per group) had electrodes located in the shell of the nucleus accumbens and were removed from electrochemical analyses.

ACKNOWLEDGMENTS. We thank Scott Ng-Evans for considerable technical assistance and Ingo Willuhn for helpful comments. This research was supported by National Institutes of Health Grants F31-AA018053 (to N.A.N.), F32-DA024540 (to J.J.C.), R21-AG30775 and R01-MM79292 (to P.E.P.), and R01-DA014609 (to I.L.B.) and by the Alcohol and Drug Abuse Institute (N.A.N. and J.J.C.).

1. Nutt DJ, King LA, Phillips LD, Independent Scientific Committee on Drugs (2010) Drug harms in the UK: A multicriteria decision analysis. *Lancet* 376:1558–1565.
2. National Institute on Alcohol Abuse and Alcoholism (2009) *Make a Difference: Talk to Your Child About Alcohol* (Natl Inst Health, Bethesda) NIH Publ No 06-4314.
3. Chambers RA, Taylor JR, Potenza MN (2003) Developmental neurocircuitry of motivation in adolescence: A critical period of addiction vulnerability. *Am J Psychiatry* 160:1041–1052.
4. Bechara A, Damasio H (2002) Decision-making and addiction (part I): Impaired activation of somatic states in substance dependent individuals when pondering decisions with negative future consequences. *Neuropsychologia* 40:1675–1689.
5. Nasrallah NA, Yang TWH, Bernstein IL (2009) Long-term risk preference and suboptimal decision making following adolescent alcohol use. *Proc Natl Acad Sci USA* 106:17600–17604.
6. Cardinal RN, Howes NJ (2005) Effects of lesions of the nucleus accumbens core on choice between small certain rewards and large uncertain rewards in rats. *BMC Neurosci* 6:37.
7. Kuhnen CM, Knutson B (2005) The neural basis of financial risk taking. *Neuron* 47: 763–770.
8. Di Chiara G, Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci USA* 85:5274–5278.
9. Budygin EA, et al. (2007) Effects of chronic alcohol exposure on dopamine uptake in rat nucleus accumbens and caudate putamen. *Psychopharmacology (Berl)* 193: 495–501.
10. Ljungberg T, Apicella P, Schultz W (1992) Responses of monkey dopamine neurons during learning of behavioral reactions. *J Neurophysiol* 67:145–163.
11. Stuber GD, et al. (2008) Reward-predictive cues enhance excitatory synaptic strength onto midbrain dopamine neurons. *Science* 321:1690–1692.
12. Fiorillo CD, Tobler PN, Schultz W (2003) Discrete coding of reward probability and uncertainty by dopamine neurons. *Science* 299:1898–1902.
13. Gan JO, Walton ME, Phillips PE (2010) Dissociable cost and benefit encoding of future rewards by mesolimbic dopamine. *Nat Neurosci* 13:25–27.
14. Platt ML, Huettel SA (2008) Risky business: The neuroeconomics of decision making under uncertainty. *Nat Neurosci* 11:398–403.
15. Floresco SB, Whelan JM (2009) Perturbations in different forms of cost/benefit decision making induced by repeated amphetamine exposure. *Psychopharmacology (Berl)* 205:189–201.
16. Preuschoff K, Bossaerts P, Quartz SR (2006) Neural differentiation of expected reward and risk in human subcortical structures. *Neuron* 51:381–390.
17. Day JJ, Jones JL, Wightman RM, Carelli RM (2010) Phasic nucleus accumbens dopamine release encodes effort- and delay-related costs. *Biol Psychiatry* 68:306–309.
18. Phillips PE, Walton ME, Jhou TC (2007) Calculating utility: Preclinical evidence for cost–benefit analysis by mesolimbic dopamine. *Psychopharmacology (Berl)* 191: 483–495.
19. Redish AD, Jensen S, Johnson A (2008) A unified framework for addiction: Vulnerabilities in the decision process. *Behav Brain Sci* 31:415–437, discussion 437–487.
20. Crews F, He J, Hodge C (2007) Adolescent cortical development: A critical period of vulnerability for addiction. *Pharmacol Biochem Behav* 86:189–199.
21. Fiorino DF, Phillips AG (1999) Facilitation of sexual behavior and enhanced dopamine efflux in the nucleus accumbens of male rats after D-amphetamine-induced behavioral sensitization. *J Neurosci* 19:456–463.
22. Wyvell CL, Berridge KC (2001) Incentive sensitization by previous amphetamine exposure: Increased cue-triggered "wanting" for sucrose reward. *J Neurosci* 21: 7831–7840.
23. Bickel WK, Marsch LA (2001) Toward a behavioral economic understanding of drug dependence: Delay discounting processes. *Addiction* 96:73–86.
24. Schultz W (2009) Midbrain dopamine neurons: A retina of the reward system? *Neuroeconomics: Decision Making and the Brain*, ed Glimcher PW (Academic, San Diego), pp 321–328.
25. Kelley AE, Berridge KC (2002) The neuroscience of natural rewards: Relevance to addictive drugs. *J Neurosci* 22:3306–3311.
26. Roitman JD, Roitman MF (2010) Risk-preference differentiates orbitofrontal cortex responses to freely chosen reward outcomes. *Eur J Neurosci* 31:1492–1500.
27. Frank MJ, Seeberger LC, O'Reilly RC (2004) By carrot or by stick: Cognitive reinforcement learning in parkinsonism. *Science* 306:1940–1943.
28. Clark JJ, et al. (2010) Chronic microsensors for longitudinal, subsecond dopamine detection in behaving animals. *Nat Methods* 7:126–129.
29. Rowland NE, Nasrallah N, Robertson KL (2005) Accurate caloric compensation in rats for electively consumed ethanol-beer or ethanol-Polycose mixtures. *Pharmacol Biochem Behav* 80:109–114.
30. Peris J, et al. (2006) Brain ethanol levels in rats after voluntary ethanol consumption using a sweetened gelatin vehicle. *Pharmacol Biochem Behav* 85:562–568.
31. Li Z, et al. (2008) High temporal resolution of amino acid levels in rat nucleus accumbens during operant ethanol self-administration: Involvement of elevated glycine in anticipation. *J Neurochem* 106:170–181.
32. Heien ML, et al. (2005) Real-time measurement of dopamine fluctuations after cocaine in the brain of behaving rats. *Proc Natl Acad Sci USA* 102:10023–10028.
33. Paxinos G, Watson C (2005) *The Rat Brain in Stereotaxic Coordinates* (Academic, San Diego), 5th Ed.