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A single exposure to voluntary ethanol self-administration produces adaptations in ethanol consumption and accumbal dopamine signaling

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Abstract

In well-trained animals, accumbal dopamine release is stimulated during operant ethanol self-administration, but the time course of development of this dopaminergic response, particularly during the acquisition of ethanol drinking behavior, remains unknown. To examine this, we trained male Longe–Evans rats to self-administer 10% ethanol plus 10% sucrose, using a protocol in which the concentration of ethanol was kept constant throughout the study. The animals were required to press the lever four times to gain continuous access to the drinking solution for 20 minutes, and microdialysis was performed on either the first or second day of 10% ethanol plus 10% sucrose self-administration or 10% sucrose as controls. Ethanol and dopamine were both analyzed in the dialysates. All groups (day 1 and 2 ethanol and their corresponding sucrose controls) showed an increase in accumbal dopamine during the transfer from the home cage into the operant chamber. Our main finding was an increase in dopamine in the nucleus accumbens core–shell border during the first 5 minutes of consumption on the second day but not on the first day of ethanol self-administration. Our results suggest that a single exposure to a 10% ethanol plus 10% sucrose drinking solution may be sufficient to learn the association between ethanol cues and its reinforcing properties. Furthermore, we speculate that the dopamine response during ethanol consumption likely reflects the reward-prediction role of the mesolimbic dopamine system.

Keywords

Nucleus accumbens; Dopamine; Self-administration; Microdialysis; Ethanol; Initiation

Introduction

Mesolimbic dopamine transmission is implicated in various aspects of reward processing, including the formation of associations between a reward and the stimulus cues that predict the reward. If an animal is exposed to an unexpected reward, the firing rate of dopamine neurons is enhanced and extracellular dopamine concentrations are elevated in terminal regions, such as the nucleus accumbens (Day et al., 2007; Schultz 1998, 2007). However, in Pavlovian and instrumental conditioning experiments, repeated pairing of a cue with a receipt of a food reward produces an increase in dopamine neuronal activity at the time at which the cue is presented (Day et al., 2007; Roitman et al., 2004; Schultz 2010). Similarly, the association between a cue and drug reward or intracranial self-stimulation is

accompanied by a temporal shift in accumbal dopamine concentrations, as assessed by fast-scan cyclic voltammetry (Aragona et al., 2009; Owesson-White et al., 2008). The number of trials required to produce the temporal shift in dopamine activity during operant conditioning is typically at least 30, and the shift has been demonstrated to take place within 1 day (Aragona et al., 2009; Stuber et al., 2008). However, no one has investigated the time course of the association between reward and cues for ethanol self-administration or potential adaptations in accumbal dopamine signaling during the initial stages of ethanol reinforcement.

Previous work from our laboratory showed that extracellular accumbal dopamine increases transiently (~5 min) at the commencement of consumption of ethanol in a limited-access operant self-administration model after extensive (months) experience with ethanol (Doyon et al., 2003). We later found that this dopamine response pattern is apparent after only 6 days of ethanol intake (Doyon et al., 2005). Interestingly, the increase in dopamine occurs before peak brain ethanol concentrations have been achieved. The accumbal dopamine response during ethanol consumption is instead consistent with the idea that the sensory stimuli of ethanol (taste and smell) serve as cues that predict the intoxicating effects of ethanol. These results show that mesolimbic dopamine signaling is enhanced during ethanol consumption in rats that have experienced the intoxicating and presumably the rewarding effects of ethanol compared with control rats that consume sucrose or water. However, no one has studied the development of the dopaminergic response or its relationship with changes in ethanol consumption that occur during the initiation of ethanol self-administration.

In addition, the nucleus accumbens is composed of different subregions, including the core, the shell, and the core-shell border. The core-shell border is the region between the core and the shell of the nucleus accumbens, but this has not been well defined using anatomical markers. There are only a handful of studies that have targeted the core-shell border as the region of interest. Recently, we showed that there is an increase in dopamine during the first 5 minutes of 10% ethanol plus 10% sucrose operant self-administration in the core-shell border of the nucleus accumbens but not in the shell or core (Howard, et al., 2009). Therefore, we decided to make the core-shell border the region of interest in this study.

To further define the time course of adaptations in mesolimbic dopamine function that accompany the development of ethanol reinforcement, we used a novel ethanol self-administration protocol in which rats are switched from a 10% sucrose solution to one containing 10% sucrose plus 10% ethanol (Carrillo et al., 2008). Ethanol intake increases significantly after the first day of ethanol exposure (Carrillo et al., 2008). Therefore, we hypothesized that neuroadaptations in dopamine signaling within the nucleus accumbens core-shell border would also occur during this period. Here, we show that a single exposure to ethanol in a drinking solution is sufficient to produce both an enhanced accumbal dopamine response and enhanced ethanol consumption 24 h later.

Materials and methods

Animals

We used forty male Long-Evans rats (Charles River Laboratories, Wilmington, MA) for this study. The animals were handled and weighed for a minimum of 5 days before performing surgery on them. Each rat was housed individually in a temperature (25°C) and light (12 h light/12 h dark) controlled environment. The rats had food and water available ad libitum in their home cage. All procedures complied with guidelines specified by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of the University of Texas at Austin.

Behavioral Apparatus

Operant chambers (Med Associates Inc., St. Albans, VT) modified for microdialysis were used for self-administration training and microdialysis testing. A retractable lever was located on the left side of one wall, (2 cm above the floor) which triggered the entry of a retractable drinking spout on the right side of the same wall (5 cm above the grid floor) when pressed. The opposite wall contained an interior chamber light. The floor was composed of parallel stainless steel bars, which connected with the spout of the drinking bottle to form a lickometer circuit (Med Associates Inc.). The operant chamber was housed within a sound-attenuating chamber with a fan, but the doors were removed to facilitate training and microdialysis. The interior light and sound-attenuating fan were activated at the beginning of each operant session. Operant chamber function and acquisition of lickometer data were controlled by PC using software from Med Associates.

Surgery

We surgically inserted a stainless steel guide cannula (21 gauge; Plastics One Inc., Roanoke, VA) above the left nucleus accumbens core-shell border using a stereotaxic device. The coordinates were (in millimeter relative to bregma): +1.7 anteroposterior, +1.0 or 1.1 lateral, -4.0 or -3.8 ventral to the skull surface (Paxinos et al., 1999). The surgical procedure used in our laboratory has been previously described (Doyon et al., 2005). The rats were given a week to recover from surgery before starting their operant training.

Self-administration training

Operant sessions occurred once a day for 7 days/wk. Animals were trained to lever press for access to a 10% sucrose (wt/vol) solution. Rats were water deprived (15–22 h/d) before each training session to facilitate learning of the operant response. A reliable lever-pressing response for the drinking solution occurred within 1–3 days; and once established, the rats were given water ad libitum for the remaining sessions. The rats were then trained to lever press for either 10% sucrose or 10% ethanol (wt/vol) plus 10% sucrose (wt/vol) as previously described by Carrillo et al. (2008). Briefly, all rats had four sessions of 10% sucrose self-administration. During the first four sessions, we gradually habituated the animals to a 15-min wait period, which preceded access to the lever and drinking solution. The lever-pressing requirement was progressively increased to four during the first four sessions as well. When the response requirement was completed, the lever was retracted, and the rats had access to the drinking solution for 20 min. Following this period, the drinking spout retracted, and the rats remained in their operant chambers for another 20 min postdrink period without the lever or drinking solution. After the fourth session, the ethanol groups were switched to a 10% ethanol plus 10% sucrose drinking solution, and microdialysis occurred on either the first or second day of ethanol exposure. Sucrose controls continued to self-administer 10% sucrose throughout the study and microdialysis was performed on equivalent days to their ethanol-consuming counterparts. Consumption of the drinking solutions was monitored throughout the self-administration protocol by a lickometer circuit and by measuring the volume of liquid in the drinking bottle before and after the session (with a resolution of 0.1 mL), accounting for spillage. Body weights were recorded every day.

Microdialysis

The microdialysis probes were constructed according to the methods described by Pettit and Justice (1991) (2.0 mm active membrane length, 270 mm OD, 18,000 molecular weight cutoff). The animals were habituated to the microdialysis tethering apparatus by tethering the rats 2 days before the microdialysis procedure until the end of the experiment. The day before microdialysis, we implanted a probe through the guide cannula (while lightly

anesthetized under 2% isoflurane) and perfused the microdialysis probes with artificial cerebral spinal fluid (2.8 mM KCl, 149 mM NaCl, 1.2 mM CaCl₂, 1.2 mM MgCl₂, 5.4 mM D-glucose, and 0.25 mM ascorbic acid) at a flow rate of 2 μ L/min (CMA 100 microinjection pump, Microdialysis, Acton, MA). After the animals recovered from the anesthesia, the flow rate was decreased to 0.2 μ L/min overnight. After stabilizing overnight, the flow rate was returned to 2.0 μ L/min, 2 h before commencing the microdialysis procedure. We manually changed the samples and 2 μ L were pipetted into a 2 mL glass vial for further ethanol analysis, for animals that were consuming 10% ethanol plus 10% sucrose. The samples were immediately frozen on dry ice and then stored at -80°C until analyzed.

Experimental timeline and histology

Dialysis samples were taken every 5 min except during the last sample of the wait period, which included the time it took the animal to lever press (Fig. 1). Four samples were collected during a baseline period in the home cage. One sample was collected while the animal was transferred into the operant chamber from its home cage and 3 samples were collected while the animals waited for the drinking solution. The last of these 3 samples also included lever pressing. Once the response requirement was completed, the lever retracted and the drinking solution was presented and remained inside the chamber for 20 min. After the 20 min, the bottle was retracted and the animal remained in the chamber for another 20 min in the absence of the drinking solution. After the experiment was completed, the probe track was mapped using the procedures previously described by Howard et al. (2009).

Dopamine analysis by high-pressure liquid chromatography

Reversed-phase high performance liquid chromatography (HPLC) with electrochemical detection was used to analyze dialysate dopamine. Seven microliter of the dialysate were mixed with 1.5 μ L of ascorbate oxidase before injection, and 5–7 μ L of this mixture was injected into the system with an 8125 manual injector (Rheodyne, Cotati, CA). Separation of dopamine occurred with one of several C18 columns used during the course of these experiments (Luna 50 $\times$ 1.0 mm, 3- μ m particle size; Phenomenex, Torrance CA; 50 $\times$ 0.5 mm column; Higgins analytical, Mountain View, CA; or 50 $\times$ 2.0 mm column; Varian, Palo Alto, CA). Detection of dopamine took place with either a VT-03 flow cell with a 2 or 0.7 mm diameter glassy carbon working electrode (ISAAC reference, Antec Leyden, The Netherlands) or a SenCell flow cell with a 2 mm electrode. Mobile phase had a pH of 5.6 and consisted of 0.50 g octanesulfonic acid, 0.05 g decanesulfonic acid, 0.13 g ethylenediaminetetraacetic acid, 11.08 g NaH₂PO₄, 4.47 g KCl, and 150 mL methanol in 1 L of deionized water. EZChrom software (Scientific Software Inc., Pleasanton, CA) was used to record and analyze chromatograms. External dopamine standards were used to determine the concentration of dopamine in each sample. The signal to noise ratios were calculated and recorded for all samples, and only dopamine peaks with a signal to noise ratio above 10 were included in the analyses.

Ethanol analysis by gas chromatography

A detailed description of the methods used for ethanol analysis is described by Doyon et al. (2003). Briefly, during ethanol self-administration, 2 μ L of dialysate collected from each sample was put into a glass vial and used for ethanol analysis. This was conducted using a gas chromatograph (Varian CP 3800; Varian, Walnut Creek, CA) with flame ionization detection (220 $^{\circ}$ C).

Statistics

Two phases of the experiment were analyzed separately: effect of transfer into the operant chamber and effect of consumption of ethanol. Initially, we analyzed the home cage

dialysate dopamine concentrations for stability using the criterion that the relative standard deviation had to be less than 0.2. One rat did not meet this criterion and was excluded from the transfer effect analysis. Another rat was excluded because the increase in dopamine during the transfer period was an outlier (Q-test). A third rat was excluded because of lost samples during the baseline and wait phases of the experiment. A total of 6 samples, of 640, were excluded because of problems with HPLC analysis. We therefore averaged adjacent time points to estimate these values and then adjusted the degrees of freedom in the ANOVA. Dialysate dopamine levels (% basal) were analyzed using a three-way ANOVA with repeated measures. For the analysis of the transfer effect, we established baseline using the four samples taken while the animals were in their home cages. We used the average of the last two wait samples to re-establish baseline for analysis of the drink effect. To perform these analyses, we used time as the within-subject factor and drinking solution and day as between-subject factors. If significant interactions were found, simple effects tests were used to determine the effect of one factor at different levels of the other factor. For between-group post hoc tests, a pooled error term was used. Dialysate ethanol levels (millimolar) were analyzed using *t*-test. Behavioral measures were analyzed using univariate ANOVAs, and if an interaction was observed, post hoc tests were run to determine the source of variation. Three behavioral parameters (number of licks, milliliter consumed, and licks during initial bout response rate) were log transformed to achieve homogeneity of variance. The number of bouts, a period of at least 25 licks with no more than 2 min between licks, was analyzed using the nonparametric Mann Whitney *U*Test. Significance for all analyses was determined when *P*-value was less than .05, and Bonferroni corrections were used for post hoc tests.

Results

The consumption of ethanol was significantly greater on the second day of exposure to the 10% ethanol plus 10% sucrose solution compared with the first day. Two pieces of evidence support this contention. The first pertains to the dose of ethanol consumed during the microdialysis days (0.38 ± 0.06 g/kg for the first day, 1.3 ± 0.15 g/kg for the second day, $P < .05$ by *t*-test). The second analysis was a comparison of the ethanol dose consumed during the first and second day, but within the group that underwent microdialysis on day 2 only (0.63 ± 0.07 g/kg for the first day, 1.3 ± 0.15 g/kg for the second day, $P < .05$ by paired *t*-test). The increase in consumption was confirmed by measurement of peak dialysate ethanol concentrations which were significantly higher ($P < .05$, *t*-test) in the day 2 ethanol group (1.7 ± 0.3 mM) compared with the day 1 ethanol group (0.4 ± 0.3 mM). However, ethanol concentrations peaked in the brain at approximately the same time in both groups, about 30 min after ethanol consumption had commenced (Fig. 2). The volume of 10% sucrose consumed was similar across these two corresponding days for the control group (Table 1). The increased consumption of the ethanol solution during the second day of exposure compared with the first was also corroborated by analysis of the lickometer data, which indicated higher values for total number of licks during the session, number of licks during the initial bout, initial bout response rate, and number of bouts (Table 1). A large majority of licking was observed in the first 5 min of fluid consumption for all groups (day 1 and 2 ethanol and the sucrose controls). For the day 2 ethanol group, the total licks within the first 5 min of self-administration were $73 \pm 7\%$ and this dropped to $16 \pm 6\%$, $6 \pm 3\%$, and $4 \pm 2\%$ in the next three 5-min periods. The day 1 ethanol group and the two sucrose control groups had similar licking patterns (day 1 ethanol: $78 \pm 7\%$ in the first 5 min and $9 \pm 5\%$, $4 \pm 2\%$, and $9 \pm 5\%$ in the following three 5-min periods; day 2 sucrose: $72 \pm 5\%$ in the first 5 min and $22 \pm 6\%$, $6 \pm 3\%$, and $0.2 \pm 0.2\%$ for the next three 5-min periods; day 1 sucrose: $68 \pm 5\%$ for the first 5 min and $26 \pm 4\%$, $2 \pm 1\%$, and $0.2 \pm 0.2\%$ in the subsequent three 5-min periods). Also, lever-press behavior during the microdialysis session was not significantly

different among the two ethanol-drinking days or the corresponding sucrose-drinking days (Table 1).

Microdialysis samples from the nucleus accumbens core–shell border were taken on the first or second day of ethanol consumption and in the sucrose controls corresponding to these days. Dialysate dopamine concentrations taken during the baseline period were not statistically different among any of the groups (1.1 ± 0.2 nM, 1.1 ± 0.1 nM for the 2 days of sucrose consumption; 0.9 ± 0.1 nM, 1.4 ± 0.2 nM for the ethanol groups). Accumbal dopamine was significantly higher during transfer from the home cages into the operant chambers (transfer period) and during the wait period compared with baseline for all groups (Fig. 3A) (main effect of time: $F[7, 226] = 16.5$, $P < .05$). Furthermore, the time course of dialysate dopamine varied across microdialysis day and drinking solution (Fig. 3B) with a more prolonged increase above baseline in the day 2 ethanol group compared with the other groups (interaction between time $\times$ day $\times$ solution: $F[7, 226] = 2.45$, $P < .05$). Post hoc analysis revealed a significant solution $\times$ day interaction for the transfer point and the first wait period ($F[1, 235] > 8.2$, $P < .05$) but not the second or third wait periods.

The average of the last two samples in the wait period was used to establish a new dialysate dopamine baseline for the analysis of accumbal dopamine during the drink and postdrink periods. Dialysate dopamine concentrations during this new baseline were not significantly different among groups (1.1 ± 0.1 nM and 1.2 ± 0.1 nM for the 2 days of sucrose consumption; 0.9 ± 0.1 nM, 1.6 ± 0.3 nM for the 2 days of ethanol consumption); (interaction between time $\times$ day $\times$ solution: $F[1, 35] = 1.1$, $P > .05$). However, we did find a significant difference in dopamine concentrations during the analysis of the drink and postdrink periods (interaction between time $\times$ day $\times$ solution: $F[9, 318] = 2.3$, $P < .05$). Post hoc analysis revealed that the day 2 ethanol group was significantly different from the day 1 ethanol group (time $\times$ day interaction for the ethanol group: $F[9, 318] = 4.3$, $P < .05$) and the day 2 sucrose group (time $\times$ solution interaction for the day 2 groups: $F[9, 318] = 2.9$, $P < .05$). Dialysate dopamine was significantly elevated above baseline in the rats that drank ethanol on the second day of exposure but not in any of the other groups of rats (Fig. 4). Post hoc tests showed a statistically significant dopamine increase during the first drink sample compared with the two baseline samples ($F[1, 318] = 29.9$, $P < .05$; $F[1, 318] = 44.7$, $P < .05$, respectively) in the day 2 ethanol group.

Calcium dependency of dialysate dopamine was $79 \pm 2\%$ ($n = 40$). All of the subjects had probes placed through the nucleus accumbens with the probes spanning the core and shell subregions but with 53% of the animals having at least 45% of the probe active area in the core–shell border of the nucleus accumbens (Fig. 5).

Discussion

This is the first study to investigate adaptations in accumbal dopamine signaling during the first 2 days of acquisition of operant ethanol self-administration. We approached this by first training rats to lever press for a solution of 10% sucrose. This was followed by exposure to either one or two consecutive days of a solution containing 10% ethanol plus 10% sucrose. On the second day of ethanol exposure, the rats more than tripled their intake compared with the rats that received ethanol on the first day, which suggests that an increase in the reward value of the solution occurred. In addition, accumbal dopamine activity was significantly enhanced during consumption of the ethanol solution on the second day but not on the first day or during sucrose consumption (Fig. 4). Therefore, a single trial of ethanol exposure is sufficient to produce neuroadaptations in accumbal dopamine signaling that are apparent 24 h after the trial.

The mechanisms that underlie the increase in ethanol consumption from day 1 to day 2 are unclear, but we propose two possibilities. Perhaps the rats became habituated to the novel aversive taste that they had previously experienced on day 1 of exposure to the ethanol plus sucrose solution. Analysis of the licking behavior supports this idea because the rats had a lower lick rate during the first ethanol-drinking bout than the rats previously exposed to ethanol or the sucrose controls. A previous study showed that habituation to aversive fluids occurs to a limited degree in Long-Evans rats (Gartside and Laycock, 1987), but this can only explain about 25% of the 200–300% increase in ethanol consumption we observed.

In conjunction with habituation to the aversive taste, we also suggest that the increase in drinking behavior across the 2 days may be due, in part, to the central pharmacological effects of ethanol experienced on the first day of ethanol consumption. Although the group of rats that was exposed to ethanol for the first time during microdialysis consumed a minimal dose (0.38 g/kg), our data indicate that ethanol does in fact reach the brain even at this dose (Fig. 2). More germane is the intake of ethanol on the first day of exposure to ethanol (0.6 g/kg) in the group in which microdialysis was done on day 2. The slightly higher consumption in this group that was not undergoing microdialysis compared with the day 1 group that did undergo microdialysis is probably explained by the mild inhibition of behavior produced by the microdialysis procedures. In any case, an ethanol dose of 0.6 g/kg is discriminable (Macenski and Shelton, 2001), and it has been shown in numerous studies to maintain lever-pressing behavior (Czachowski et al., 2001, 2003), suggesting that the dose achieved on day 1 may act as a reinforcer. Although additional studies are needed to confirm our suggestion that some rewarding sensations were experienced on the first day of ethanol consumption, the fact that consumption dramatically increased on day 2 is at least consistent with our suggestion. We further speculate that an association between the rewarding effects of ethanol and the cues present during consumption of the novel ethanol solution may have been formed. Thus, the increased ethanol intake on day 2 may, in part, be because of the enhanced motivation to seek the ethanol solution because of the newly formed association. The dopamine response may reflect the reward-prediction role of mesolimbic dopamine that developed after the single pairing between the stimulus cues of ethanol that are present during consumption and the subsequent reward produced by ethanol after a sufficient concentration has reached the brain (Horvitz, 2000; Schultz, 1998, 2007; Stuber et al., 2008). Our data are the first to show that a single exposure to voluntary ethanol self-administration is sufficient to produce adaptations in accumbal dopamine signaling.

It may be argued that part of the reason for the appearance of the accumbal dopamine response on the second day of exposure is simply because of the larger intake of ethanol along with greater brain concentrations of ethanol on that day compared with the first day. Higher brain ethanol concentrations would produce stronger and more long-lasting pharmacological effects that produce intoxication. However, in this and in previous studies, the transient dopamine response occurs before appreciable ethanol has reached the brain, and this argues against the idea that the pharmacological effects of ethanol contribute to the response that appears on day 2. Instead, the dopamine response occurs during the period of time when the rat is consuming most ethanol on the basis of the lickometer data and when the stimulus cues for ethanol are strongest (Doyon et al., 2003, 2005; Howard et al., 2009).

Furthermore, it is possible that we see an increase in dopamine during the second exposure to ethanol, but not in the sucrose controls, because the animals in the sucrose group are habituated to sucrose, whereas the animals in the ethanol group are not yet habituated to the ethanol. The sucrose animals had 4–5 days of exposure to the sucrose solution, whereas the animals in ethanol groups had 0–1 days of exposure to the ethanol plus sucrose solution. However, previous work in our laboratory has shown that exposure to ethanol solutions for 7 days or 7 weeks does not produce habituation of the dopamine response observed during

ethanol consumption (Doyon et al., 2003, 2005). On the other hand, evidence suggests that a dopamine response during sucrose consumption may occur early during operant training (Doyon et al., 2004), but that habituation of this response does occur with continued operant self-administration (present results, Doyon et al., 2005; Howard et al., 2009). Therefore, the dopamine response seen during ethanol self-administration does not habituate, whereas habituation of the dopaminergic response appears to take place with sucrose self-administration.

Alternatively, differences in the nature and time course of reward produced by sucrose and ethanol consumption could explain the lack of dopamine response during sucrose consumption, although sucrose is clearly a strong reinforcer. The taste of sucrose is likely to be rewarding to the rats, whereas the pharmacological effects and not the taste of ethanol is what the rats appear to find rewarding. It has been shown that after repeated pairings between a reward and a cue, the dopamine neuronal activity in the ventral tegmental area shifts from the time that the reward is received to the time that the cue occurs (Schultz, 1998, 2007; Schultz et al., 1997). In the case of sucrose reward, the dopamine response may have shifted from the time of consumption to the proximal cue of being transferred into the operant chamber. However, with ethanol consumption, the stimulus cues during consumption predict the pharmacological reward, and therefore, the dopamine response occurs during consumption.

It should be noted that the animals were drinking a sweetened ethanol solution. This can make it difficult to determine if the results are because of the effects of ethanol itself or to ethanol enhancing the rewarding properties of sucrose. However, because we are examining the early stages of ethanol self-administration and rats find ethanol aversive, we cannot divorce the sucrose and ethanol solutions. Regardless, our findings still show that whether it is the rewarding properties of ethanol itself or ethanol enhancing the rewarding properties of sucrose, ethanol clearly contributes to the development of the dopaminergic response we see during the consumption of 10% ethanol plus 10% sucrose. This is reinforced by the lack of increase in accumbal dopamine when the animals self-administer 10% sucrose only.

We should also note that a limitation of our study is that we only see this significant increase in dopamine levels from baseline during the first sample of the drink period (day 2 ethanol group). Additional studies to further define the time course at a higher resolution are warranted. However, the present results are consistent with previous studies in our laboratory that have shown a transient increase in dopamine during the first 5–10 min of ethanol consumption that quickly comes back down to baseline levels (Doyon et al., 2003, 2005; Howard et al., 2009).

An increase in dialysate dopamine within the nucleus accumbens core–shell border was also observed during the transfer from the home cage into the operant chamber (transfer period), and the time course of this effect varied with respect to the day of ethanol exposure and the drinking solution. The dialysate dopamine concentration increased above baseline followed by a drop during the transfer and first wait period for three of the groups (the two sucrose groups and the day 1 ethanol group), whereas the initial enhancement in dopamine was sustained for the next 5 min in the day 2 ethanol group (Fig. 3B). Overall, the present data provide evidence that suggests that dopamine signaling has begun to change in ethanol-exposed rats even during exposure to the cues associated with the operant chamber after a single day of exposure to ethanol but not in control rats. Additional work will be required to verify this suggestion.

In summary, the main finding of this study is that there is a transient increase in dopamine within the nucleus accumbens core–shell border during the first 5 min of 10% ethanol plus

10% sucrose self-administration on the second day of ethanol exposure. This study gives us new insight that the development of the neurochemical and behavioral changes during the acquisition of ethanol self-administration occurs quite rapidly after a single pairing trial. More specifically, these results are consistent with the proposed role of accumbal dopamine as a reward-prediction signal during the development of voluntary ethanol consumption, furthering our understanding of the relationship between mesolimbic dopamine and ethanol reinforcement.

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Fig. 1.

Experimental timeline for microdialysis session. Phases include: baseline; transfer; wait; and final 5-min wait period, including time to complete response requirement, drink, and postdrink. Numbers indicate the time in minutes for each phase. The variability of the final wait period is because of the variability in the time it takes for the rat to complete the response requirement.

Fig. 2. Brain ethanol concentrations during operant ethanol self-administration. Dialysate ethanol concentrations from the nucleus accumbens core-shell border during the drink and postdrink periods on the first ($n = 12$) and second days of ethanol self-administration ($n = 10$).

Fig. 3.

Accumbal dopamine during the baseline, transfer, and wait periods. (A) All groups are collapsed and presented as one. Arrows indicate the time of transfer from home cage into operant chamber and time during which the rat had access to the lever. Asterisks indicate $P < .05$ for dopamine when compared with the home cage baseline when all groups are collapsed: both days of 10% ethanol plus 10% sucrose or 10% sucrose ($n = 37$). (B) Each group is presented individually. For clarity only, representative error bars for each time period are shown. Plus signs indicate a significant day $\times$ solution interaction ($P < .05$) at the indicated time points.

Fig. 4.

Accumbal dopamine during operant ethanol or sucrose self-administration. (A) Dialysate dopamine from the nucleus accumbens core-shell border during the wait, drink, and postdrink periods on the second day of exposure to ethanol ($n = 10$) and corresponding sucrose controls ($n = 8$). The arrow indicates the time during which the animal had access to the lever. Mean $\pm$ standard error of the mean are shown. The asterisk indicates a significant increase $P < .05$ for dopamine during the first drink period, when compared with the wait period (new baseline). (B) Data collected from the first day of 10% ethanol plus 10% sucrose ($n = 12$) and the corresponding day for the 10% sucrose group ($n = 10$).

Fig. 5.

Schematic representation of probe placements in the nucleus accumbens core-shell border for all groups. Groups include: day 2 and day 1 ethanol self-administration and the corresponding sucrose groups. Numbers above slices denote the location in millimeter from bregma. This figure was based on the rat brain atlas of Paxinos et al. (1999). The light gray shading indicates the core. The dark gray shading indicates the core-shell border, and the white area of the accumbens indicates the shell subregion.

Table 1

Behavioral parameters on microdialysis day

Parameter	Day 1		Day 2	
	Sucrose	Ethanol + Sucrose	Sucrose	Ethanol + Sucrose
Latency to begin drinking (min)	0.15±0.04	0.33±0.08	0.26±0.13	0.17±0.08
Number of bouts	1.10±0.10	1.67±0.19 ^a	1.38±0.18	1.65±0.18
Total licks	2339±209	362±66 ^b	2027±236	1414±224
Initial bout response rate (licks/min)	280±19	100±23 ^b	250±20	178±21
Licks during initial bout	2325±206	265±44 ^b	1948±263	1291±240
Milliliters consumed	12.0±1.0	1.8±0.3 ^b	10.3±1.1	6.3±0.8
Time to complete lever pressing requirement (min)	2.69±1.24	1.77±0.53	1.42±0.39	1.96±0.76

Values are shown as mean±standard error of the mean. A bout is a period of at least 25 licks, with no more than 2 min between licks.

^aSignificantly different from day 1 sucrose by Mann Whitney *U* test ($P<.05$).

^bSignificantly different from day 2 ethanol and day 1 sucrose by univariate ANOVA ($P<.05$).