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The dopamine response in the nucleus accumbens core-shell border differs from that in the core and shell during operant ethanol self-administration

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Abstract

Background—Ethanol self-administration has been shown to increase dopamine in the nucleus accumbens; however, dopamine levels in the accumbal subregions (core, shell, and core-shell border) have not yet been measured separately in this paradigm. The present study was designed to determine if dopamine responses during operant ethanol self-administration are similar in the core, core-shell border and shell, particularly during transfer from the home cage to the operant chamber and during consumption of the drinking solution.

Methods—Six groups of male Long-Evans rats were trained to lever-press for either 10% sucrose (10S) or 10% sucrose + 10% ethanol (10S10E) (with a guide cannula above the core, core-shell border, or shell of the accumbens). On experiment day, five-min microdialysis samples were collected from the core, core-shell border, or shell before, during, and after drinking. Dopamine and ethanol concentrations were analyzed in these samples.

Results—A significant increase in dopamine occurred during transfer of the rats from the home-cage into the operant chamber in all six groups, with those trained to drink 10S10E exhibiting a significantly higher increase than those trained to drink 10S in the core and shell. No significant increases were observed during drinking of either solution in the core or shell. A significant increase in dopamine was observed during consumption of ethanol in the core-shell border.

Conclusions—We conclude that dopamine responses to operant ethanol self-administration are subregion specific. After operant training, accumbal dopamine responses in the core and shell occur when cues that predict ethanol availability are presented and not when the reinforcer is consumed. However, core-shell border dopamine responses occur at the time of the cue and consumption of the reinforcer.

Keywords

Dopamine; Microdialysis; Operant Ethanol Self-Administration; Core; Core-Shell Border; Shell; Nucleus Accumbens

INTRODUCTION

Extracellular dopamine concentrations in the nucleus accumbens increase during operant ethanol self-administration, but the precise function of the stimulation of accumbal dopamine signaling during ethanol reinforced behavior is still debated (Doyon et al., 2005; Doyon et al., 2003; Gonzales and Weiss, 1998; Melendez et al., 2002; Weiss et al., 1993).

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The core and shell subregions of the nucleus accumbens are anatomically distinct from one another (Heimer et al., 1991; Zahm and Brog, 1992; Zahm, 1999), but it is unclear whether core or shell dopamine contributes to the observed response during ethanol self-administration. Furthermore, dopamine in the nucleus accumbens core and shell may have different behavioral functions. For example, dopamine in the core of the nucleus accumbens has been implicated in associative conditioning and instrumental behaviors (Bassareo and Di Chiara, 1999a; Day et al., 2007; Ito et al., 2000; Sokolowski and Salamone, 1998), whereas dopamine in the shell may be important during exposure to novel or conditioned stimuli or the acquisition of place preference (Bassareo and Di Chiara, 1999b; Cheng et al., 2003; Fenu et al., 2006; Rebec et al., 1997). In addition, dopamine signaling in the core and shell differs during reinstatement of heroin self-administration (Bossert et al., 2007), operant responding for food reward (Sokolowski et al., 1998), and acute non-contingent intravenous ethanol administration (Howard et al., 2008). However, the potential differential changes in dopamine levels in the core and shell during operant ethanol self-administration have not been defined.

More recently, studies have started to investigate dopamine in the border between the core and shell subregions, known as the “shore”. Little is known about the behavioral functions of dopamine in the core-shell border of the accumbens and the anatomical connections of this area. However, dopamine in this area has been reported to respond differently from that in the core and shell to novelty and mu and delta opioid agonists (Hipolito et al., 2008; Rebec et al., 1997). No studies investigating ethanol’s effects on dopamine in the core-shell border have been published, and more work is needed to define the role of dopamine in this area in ethanol reinforcement.

It is also unclear if the dopamine increase observed in the accumbens during operant ethanol self-administration is due to the pharmacological effects of ethanol or environmental cues associated with its administration. It has been well established that dopamine increases in the accumbens during acute ethanol administration in naïve animals (Blomqvist et al., 1993; Campbell and McBride, 1995; Howard et al., 2008; Imperato and Di Chiara, 1986; Kohl et al., 1998; Tang et al., 2003; Yim et al., 2000; Yoshimoto et al., 1992a; Yoshimoto et al., 1992b). In contrast, previous studies from our lab indicated that the time course of the increase in accumbal dopamine did not match that of brain ethanol suggesting that the increase in dopamine was more physiological rather than pharmacological (Doyon et al., 2003). After associative conditioning between a cue and reinforcer, the cues that predict a reinforcer may increase dopamine in the accumbens. For example, Schultz et al. (1997) showed that after repeated training sessions, stimulation of dopamine cell firing rate changed from the presentation of the unpredicted reward to the presentation of the cue that predicted the subsequent reward availability. Also, Stuber et al. (2008) reported that cue-evoked dopamine release in the nucleus accumbens developed over the course of the learning of the association between the cue and the subsequent reward. However, these studies did not compare the dopamine responses in core, core-shell border, and shell of the accumbens. In order to further define the role of dopamine in ethanol reinforcement, it is important to investigate whether dopamine levels increase as a result of pharmacological effects or environmental cues, and if this response is the same in the accumbal subregions.

The major goal of this study was to determine whether dopamine in the core and shell of the nucleus accumbens would respond in a similar manner during operant ethanol self-administration, and if dopamine in the core-shell border would respond in a similar manner to the core or shell. A secondary goal was to investigate dopamine levels in these three accumbal subregions during transfer from the home-cage into the operant chamber, when environmental stimuli associated with operant ethanol self-administration are introduced.

MATERIALS AND METHODS

Subjects

Forty-seven male Long-Evans rats (Charles River Laboratories, Wilmington, MA) were included in the analyses for these experiments. The rats were housed individually in a temperature (25°C) and light (12 hour light/12 hour dark) controlled room, and had access to food and water *ad libitum*. The rats were handled and weighed for at least five days prior to surgery. All procedures were carried out in compliance with the guidelines set forth by the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of the University of Texas at Austin.

Surgery

Prior to operant training and testing, a stainless steel guide cannula (21 gauge, Plastics One Inc., Roanoke, VA) was placed in each rat above either the left nucleus accumbens core (coordinates in mm relative to bregma: AP +1.3, ML +1.6, DV -3.2), core-shell border (AP +1.7, ML +1.2, DV -3.8), or shell (AP +2.2, ML +0.7, DV -3.8) while the animal was in a stereotaxic frame. The DV coordinate represents the bottom of the guide cannula, and the microdialysis probe extended an additional 4.0 mm below the cannula when seated into the guide. The guide cannula and a single steel bolt used for tethering the animal during microdialysis were cemented to the skull using dental cement (Plastics One Inc.). An obturator was placed in the guide cannula to prevent blockage during training. The rats were under isoflurane anesthesia (4.0 % during the induction period and 2.0 % during maintenance) during surgery. Rats were allowed to recover from surgery for at least one week before training.

Behavioral Apparatus

Operant chambers (Med Associates Inc., St. Albans, VT) modified for microdialysis were used for self-administration training and testing. One wall of each chamber contained a retractable lever on the left side (2 cm above stainless steel bar floor) and a retractable drinking spout on the right side (5 cm above floor), while the opposite wall contained an interior chamber light. The bars which made up the floor were connected to the metal spout via a lickometer circuit (Med Associates). The operant chamber was housed within a sound-attenuating chamber with a fan; however, the doors were removed to facilitate training and microdialysis. At the start of each operant session, the interior light and sound-attenuating fan were activated. Computer software (Med Associates) controlled operant chamber function and acquisition of lickometer data.

Self-Administration Training

Operant sessions occurred once a day for 5 days per week. Rats were trained to lever-press for access to 10S (w/v). Animals were water deprived (<22 hr / day) prior to each session (30 – 45 min) to facilitate learning of this operant response. Reliable lever-pressing occurred within 2 – 4 days, and for the remainder of the study, rats were given water *ad libitum*. After the rats were trained to lever-press, half of the subjects were trained to self-administer 10% ethanol plus 10% sucrose (10S10E) using a modified sucrose fading procedure in which the sucrose was not faded out (Samson, 1986). We increased the concentration of ethanol (w/v) in the drinking solution across sessions (2–10% over 6 days), but did not remove the sucrose from the drinking solution (Table 1). The other half of subjects self-administered 10% sucrose (10S) over the same number of days as the 10S10E group. During the modified sucrose fading procedure leading up to the dialysis experiment, we also gradually habituated the rats to a 15 min wait period, which preceded presentation of the lever, and a response

requirement of 4 lever-presses (Table 1). For each session, completion of the response requirement retracted the lever and led to presentation of the drinking spout for 20 min. The spout then retracted for 20 min of post-drinking time. In previous studies (Doyon et al., 2005; Doyon et al., 2003), some rats developed an ethanol aversion after drinking large doses when the ethanol concentration in the drinking solution reached 5 or 10%. In the present study, after one rat developed an aversion to ethanol, we tried to prevent this by limiting the volume of ethanol consumed during the training to 15 ml for 5% ethanol and 7 ml for 10% ethanol. However, during the day of microdialysis, the rats were not limited and had access to 20 ml. The 10S groups were never exposed to ethanol. Self-administration parameters were monitored during training and microdialysis by a lickometer circuit. Milliliters of drinking solution consumed and body weights were recorded each day.

Microdialysis

Habituation to the microdialysis tethering apparatus occurred during the two days prior to microdialysis. Rats were tethered using brief (~5 min) 2% isoflurane anesthesia and left overnight in the testing room, with continued tethering during the operant session the following day. Immediately after this session, animals were again briefly anesthetized (10–15 min) using 2% isoflurane to allow the probe to be slowly placed in the guide cannula.

The probes were constructed in the laboratory according to the methods of Pettit and Justice (1991) (1.5 mm active membrane length, 270 μ m OD, 18,000 molecular weight cut-off) and perfused (CMA 100 microinjection pump, Acton, MA) with artificial cerebrospinal fluid (ACSF: 149 mM NaCl, 2.8 mM KCl, 1.2 mM MgCl₂, 1.2 mM CaCl₂, 0.2 mM ascorbic acid, 5.4 mM D-glucose). After probe implantation, rats were placed in individual cages with free access to water and food, and the flow rate was lowered to 0.2 μ l/min overnight. After a stabilization period of 12–15 hours, the flow rate was increased to 2.0 μ l/min, and two hours were allowed at this new flow rate before sample collection commenced using five-min intervals. Samples were manually changed, 2 μ l were pipetted into a 2 ml glass vial for ethanol analysis if the animal was consuming 10S10E, and then immediately frozen on dry ice. The samples were stored at –80° C until analyzed.

Experimental Timeline

Dialysis samples were taken every five minutes except for the last wait-period sample as indicated below. Four basal samples were collected while the animal was still in the home-cage placed beside the operant chamber. One sample was collected after the animal was transferred from the home-cage to the chamber. The operant program started after the transfer period sample was taken. Three samples were then taken prior to presentation of the drinking spout: two samples during the wait period, and the third including the variable lever-pressing time (approximately 5.2 minutes total). When the drinking spout entered the chamber, the first five-minute drink sample began. In this way, any increases in dopamine resulting from responding were included in the third wait sample, and increases due to consumption started in the first drink sample. Completion of the response requirement was followed by a 20 min drinking period (four samples) and then a 20 min post-drinking period (four samples). At the end of the operant program, the rat was transferred back to the home-cage. Then the perfusate was switched to calcium-free ACSF. A five-minute sample was taken after one and a half hours to verify that dopamine recovered in the experimental samples was due to exocytotic release.

Histology

The day after dialysis, the animals were overdosed using an i.p. injection of sodium pentobarbital (150 mg/kg). After the animal was perfused intracardially with saline and then 10% formalin, the brain was extracted and placed in 10% formalin overnight. The brains

were sectioned (100 μ m thick) with a vibratome (Leica, Nussloch, Germany) and then stained with cresyl violet to confirm probe placement. The probe tracks were mapped using the atlas of Paxinos et al. (1999).

Dopamine Analysis by High Pressure Liquid Chromatography (HPLC)

Dialysate dopamine was analyzed using HPLC with electrochemical detection. The system used a Polaris 3 μ m C18 column (50 $\times$ 2 mm, Varian, Lake Forest, CA). The mobile phase consisted of 0.50 g octanesulfonic acid, 0.05 g decanesulfonic acid, 0.13 g ethylenediaminetetraacetic acid, 11.08 g NaH₂PO₄, and 150 ml methanol in 1 liter of deionized water. The mobile phase had a pH equal to 5.6. Seven microliters of the dialysate sample were mixed with ascorbate oxidase at 4° C prior to injection. Dopamine was detected with an electrochemical detector (Model VT03, Antec Leyden, Netherlands) at a potential of + 450 mV (relative to an Ag/AgCl reference). A second system was used for some samples in which the reference was an in situ Ag/AgCl (ISAAC). KCl was added to the mobile phase in appropriate concentrations in this case. The limit of detection was approximately 0.3 nM. The peaks were recorded using EZChrom software, and the concentration of dopamine in each sample was determined using external standards. The signal to noise ratios were calculated and recorded for all samples. Only animals with ratios of 3 or higher for the 0.625 nM dopamine standard and 7 or higher for the first basal sample were included in the study.

Ethanol Analysis by Gas Chromatography (GC)

Ethanol analysis was conducted according to the methods described by Doyon et al. (2003). Briefly, ethanol was analyzed in 2 μ l aliquots that were transferred into 2 ml gas chromatography vials immediately after collection of the microdialysis sample. A Varian CP 3800 gas chromatograph with flame ionization detection and a Varian 8200 headspace autosampler was used to analyze the concentrations of ethanol in the samples. The stationary phase was an HP Innowax capillary column (30.0 m $\times$ 0.5 mm $\times$ 1.0 μ m film thickness) and helium was the mobile phase. Resulting ethanol peaks were recorded using Varian Star Chromatography Workstation software, and calibration was achieved using external standards.

Statistical Analyses Performed on Data Collected from the Core and Shell of the Accumbens

Three-way analysis of variance (ANOVA) with repeated measures was used for dialysate dopamine concentrations (nM) during transfer from the home-cage to the operant chamber and during consumption of drinking solutions. The four home-cage samples served as the baseline with which the transfer and wait samples were compared, while the three wait samples served as the baseline for the drinking and post-drinking samples. For these analyses, time was the within-subject variable, and both subregion (two levels: core and shell) and drinking solution (two levels: 10S10E or 10S) were between-subject variables. Dialysate ethanol levels (mM) were analyzed using a two-way ANOVA with repeated measures. Time was the within-subject variable, and subregion was the between-subject variable. For the analysis of the time course of drinking, the percent of total licks in each of the four five-min time bins in the drink period was analyzed using a three-way ANOVA, with drinking solution and subregion as between subject variables. Behavioral parameters during operant self-administration were analyzed using multivariate ANOVA. The body weights for each of the four groups were compared using a two-way ANOVA. The between-subject variables were subregion and drinking solution. For all ANOVAs, if an interaction between the variables was observed, the simple effects were further analyzed to identify any sources of variation. The basal dopamine concentrations for core and shell were collapsed across drinking solutions, and the values were compared using a t-test. Significance for all analyses was assigned if $p < 0.05$. Post hoc tests were Bonferroni corrected.

Statistical Analyses Performed on Data Collected from the Core-Shell Border of the Accumbens

Core-shell border experiments were conducted post-hoc, and the data collected from this subregion was analyzed separately from core and shell groups. A two-way ANOVA with repeated measures was used for dopamine concentrations (time $\times$ drinking solution). Also, in order to verify that all groups experienced similar conditions basal dopamine levels, dialysate ethanol concentrations, ethanol dose consumed, behavioral parameters, and body weights were compared across core, core-shell border, and shell.

RESULTS

Body Weights and Basal Dialysate Dopamine Concentrations for Core and Shell Groups

The body weights of the rats measured on the day of the microdialysis session varied widely (300 – 460 g). To make sure that the neurochemical and behavioral analyses were not influenced by differences in body weight among the experimental groups, we analyzed the distribution of weights across groups. ANOVA indicated no significant differences in body weights between groups (core, shell, ethanol + sucrose drinkers, sucrose drinkers), because there was no significant interaction between subregion and drinking solution ($F_{1,30} = 3.6$, NS). Therefore, variation in body weight did not affect the subsequent analyses.

Dialysate dopamine concentrations in the basal samples taken in the home cage before the operant session were 1.4 ± 0.2 nM ($n = 15$) for the shell and 1.6 ± 0.2 nM ($n = 19$) for the core. These values were not significantly different from one another ($T_{32} = 0.7$, NS).

Dialysate Dopamine Concentrations after Transfer from the Home-Cage into the Operant Chamber for Core and Shell Groups

Accumbal extracellular dopamine was stimulated during the transfer from the home cage into the operant chamber in all experimental groups, and the enhanced dialysate dopamine concentration was sustained during the waiting period (Figure 1). The dialysate dopamine concentration in the first sample taken after the transfer increased over the home cage baseline with a significantly larger increase observed in the rats trained to drink ethanol + sucrose (33%) compared to those trained to drink sucrose (14–21%; $F_{6,180} = 2.2$, $p < 0.05$ for the drinking solution $\times$ time interaction). However, this dopamine response was similar in both core and shell subregions ($F_{6,180} = 0.3$, NS for subregion $\times$ time interaction).

Dialysate Dopamine Concentrations during Liquid Consumption for Core and Shell Groups

The dialysate dopamine concentrations during the wait and lever-press periods were used as a new baseline to analyze potential changes in accumbal dopamine during the drinking and post-drink periods. Overall, small, nonsignificant, increases in dialysate dopamine during the drinking period were observed in all groups (5–8% above baseline) (Figure 2; $F_{10,300} = 1.0$, NS for the drinking solution $\times$ subregion $\times$ time interaction).

Dialysate Ethanol Concentrations for Core and Shell Groups

Ethanol appeared in the dialysate within 5 min of ethanol availability for all but one of the rats that drank 10S10E. Dialysate ethanol concentrations increased over the entire course of the drink and post-drink periods, with the peak ethanol concentration reaching 2.0 ± 0.3 mM for the shell group and 1.6 ± 0.3 for the core group. The ethanol time course did not differ between core and shell (Figure 3; $F_{7,98} = 0.4$, NS for subregion $\times$ time).

Behavioral Analysis for Core and Shell Groups

The analysis of several behavioral parameters that were determined during the operant self-administration session on microdialysis day shows that the animals consuming 10S drank more than those consuming 10S10E. As a result, four consumption parameters were significantly lower for the 10S10E groups when compared to the 10S groups (collapsed across subregion): total number of licks, the duration of the first bout, the number of licks in the first bout, and the total milliliters consumed ($F_{1,30} \geq 6.4$, $p < 0.05$ for drinking solution, Table 2). Also, the 10S10E animals drank most during the first five minutes of access to the solution, while the 10S animals continued into the second five minute period. The percentage of licks in each five minute bin of the drink period differed significantly by drinking solution when collapsed across subregion (Figure 4; $F_{3,90} = 12.8$, $p < 0.05$ for drinking solution $\times$ time). Four parameters were not significantly different when compared across subregion and drinking solution: time to complete lever-press requirement, time between last lever-press and first lick, number of bouts, and the rate of licking in bout 1 ($F_{1,30} \leq 1.9$, NS for subregion $\times$ drinking solution, Table 2). Within the 10S10E drinkers, the shell group consumed 1.9 ± 0.2 g/kg ethanol, and the core group consumed 1.7 ± 0.2 g/kg, and these doses were not significantly different from one another ($T_{14} = 0.7$, NS).

Additional Analyses for Core-Shell Border Groups and Comparison to Core and Shell Groups

Because the dopamine increase observed during ethanol consumption in past studies (Doyon et al., 2005; Doyon et al., 2003) was not seen in the present core and shell groups, we performed additional experiments with probes placed on the core-shell border. A re-examination of probe placements revealed that most probes in these previous studies passed through this area, and sampled from the core-shell border as well as from the shell. Also, two missed placements from our core and shell groups that collected from the core-shell border indicated that dopamine in this subregion was increasing during consumption of ethanol (33% and 59% above baseline).

The body weights and basal dopamine levels in animals in the core-shell border groups were similar to those in the core and shell groups. The body weights measured on the day of the microdialysis were 320–440g for core-shell border animals. ANOVA showed no significant differences in body weights between all six groups (core, core-shell border, shell, ethanol + sucrose drinkers, sucrose drinkers) ($F_{2,41} = 1.9$, NS). Basal dopamine levels for core-shell border groups were 1.1 ± 0.2 nM ($n = 7$) for 10S10E drinkers and 1.2 ± 0.2 nM ($n = 6$) for 10S drinkers, and were not significantly different from basal levels in the core and shell ($F_{2,41} = 0.1$, NS).

The increase in dopamine concentration in the core-shell border during transfer of the animal into the operant chamber was similar to that observed in the core and shell groups; however, dopamine in the core-shell border during consumption responded differently from these other two areas. For core-shell border groups, the increase observed during transfer into the operant chamber in rats trained to drink ethanol + sucrose (41%) was not statistically larger than the increase observed in rats trained to drink sucrose (20%) (Figure 5; $F_{9,99} = 1.0$, NS for drinking solution $\times$ time interaction), despite the similarity of these percentages to those in the core and shell. In contrast to the core and shell groups, a significant increase in dopamine in the core-shell border was observed during consumption of either solution (Figure 6; $F_{10,110} \geq 2.3$, $p < 0.05$). However, the time course of the dopamine response differed between 10S10E and 10S drinkers ($F_{10,110} = 2.9$, $p < 0.05$, for the drinking solution $\times$ time interaction). For 10S10E drinkers, the first three samples in the drink period were significantly greater than the wait period ($F_{3,110} \geq 4.9$, $p < 0.05$), and for

the 10S drinkers, only the third sample was significantly greater than the wait period ($F_{3,110} = 5.3$, $p < 0.05$).

The increase in dopamine in the core-shell border during consumption was not due to greater ethanol concentrations reaching this area. The peak dialysate ethanol level in the core-shell border, 2.2 ± 0.5 mM, was similar to levels in the core and shell (Figure 7). The ethanol time course did not differ between core, core-shell border, and shell ($F_{14,140} = 0.4$, NS for subregion $\times$ time).

The behavioral data for the core-shell border groups was very similar to the data collected from the core and shell groups. When this data was included in the analysis, the same four consumption parameters were significantly lower for the 10S10E groups when compared to the 10S groups (collapsed across subregion): total number of licks, the duration of the first bout, the number of licks in the first bout, and the total milliliters consumed ($F_{1,40} \geq 6.6$, $p < 0.05$ for drinking solution, Table 2). Also, the percentage of licks in each five minute bin of the drink period differed significantly by drinking solution (data not shown; $F_{3,120} = 13.3$, $p < 0.05$ for drinking solution $\times$ time). Finally, this group consumed 1.9 ± 0.2 g/kg ethanol, and this dose was not significantly different from core and shell groups ($F_{2,20} = 0.4$, NS).

Histological Analysis and Calcium Dependence of Dialysate Dopamine for All Groups

Figure 8 shows the representation of the probe placements. For probes measuring from the core or shell of the accumbens, only animals with at least 80% of the probe sampling from the subregion of interest were included in the analyses. As shown in the figure, in most places the shell wraps around the core. Because of this, the shell is medial to the core and below the core. None of the probes aimed at the shell overlapped with the core, although some of the core probes dipped into the shell below the core to a limited degree. For the core analyses we only included animals with probes that penetrated the shell or core-shell border by no more than 20% of the active dialysis membrane length. In order to keep conditions similar between groups, we continued to use 1.5 mm probes for the core-shell border groups. As a result, less of the probe was within the area of interest, and our criterion for this subregion was that the probe be at least 50% within the core-shell border. For these probes, the remaining percentage sampled from the shell. Overall, the dialysate samples for all groups showed a calcium dependency of 80 ± 1 %.

DISCUSSION

This is the first study to investigate extracellular dopamine in the core, core-shell border, and shell of the nucleus accumbens during voluntary ethanol self-administration. Three major findings are reported in the present study. First, the results show that accumbal dopamine responses to operant ethanol self-administration are subregion specific. While the core, core-shell border, and shell exhibited dopamine responses during transfer from the home-cage into the operant chamber, only dopamine levels in the core-shell border increased during consumption of ethanol. The second major finding is that rats trained to drink 10S10E exhibit a significantly larger increase in accumbal dopamine in the core and shell during transfer from the home-cage into the operant chamber when compared with those trained to drink 10S. Increases in dialysate dopamine, compared with home-cage baseline values, were seen during the twenty minutes following transfer of the animal into the operant chamber, and the increase in the first five minutes was significantly higher in the 10S10E (33% above baseline) group when compared to the 10S (17% above baseline) group. The core-shell border 10S10E and 10S groups exhibited similar increases (41 & 20%, respectively). The third finding is the similarity of dopamine responses between the core and shell during all phases of this experiment. For the 10S groups dopamine increased 14–21% in the core and shell during the transfer from the home cage to the operant chamber, while in

the 10S10E groups the dopamine increased by 33% in both subregions during the transfer period. When fluid consumption started, the small change in dialysate dopamine was 5 and 8% in the first sample, in the core and shell respectively, for either consumption of 10S or 10S10E.

While the core and shell subregions have been reported to respond differently after administration of drugs of abuse, including alcohol, and receipt of food reward (Bassareo and Di Chiara, 1999a; Bossert et al., 2007; Howard et al., 2008; Sokolowski et al., 1998), we did not observe a difference in dopamine response between core and shell of the nucleus accumbens during operant ethanol self-administration. However, previous publications indicate that dopamine in both these subregions responds to the cues associated with a reinforcer. For example, dopamine in the core has been shown to respond to conditioned stimuli (Bassareo and Di Chiara, 1999a; Day et al., 2007; Ito et al., 2000), as has dopamine in the shell (Bassareo and Di Chiara, 1999b; Cheng et al., 2003). The results of the present study agree with these findings that dopamine in both subregions responds to conditioned stimuli. However, from our results we cannot conclude whether dopamine in the core and shell plays the same role during operant ethanol self-administration, or if these subregions coincidentally exhibit similar dopamine responses that have different functions.

Because the core-shell border is a newly defined subregion of the accumbens, the behavioral functions of dopamine in this area are not yet known. Dopamine in this subregion has previously been shown to respond to novelty (Rebec et al., 1997), with increases in dopamine transients being more delayed and more long-lasting than those in the shell, and no change occurring in the core. However, in the present study animals are exposed to drinking solutions and predictive cues for over a week, and therefore novelty is unlikely to contribute to the dopamine responses we observed in the core-shell border region.

This study is the first to show that rats trained to drink 10S10E have a significantly higher accumbal dopamine response when transferred from the home cage into the operant chamber compared with those trained to drink 10S. Melendez et al. (2002) reported a similar difference in accumbal dopamine between ethanol and saccharin drinkers during the waiting period before self-administration. The results of the present study agree with previous findings from our lab that enhancement of accumbal dopamine activity occurs during this phase of the experiment (Doyon et al., 2005; Doyon et al., 2003), although we previously did not observe a significant difference between the 10S and 10S10E groups. It is possible that the group sizes may not have been large enough ($n=8-11$) to reveal a difference in the previous reports. In the present study we did not find a significant interaction between drinking solution and subregion. However, we did find a main effect of drinking solution, and the group sizes were 15 and 19. Thus, the larger groups may have increased the statistical power of this analysis. It is important to note that the results of this study cannot rule out the possibility that animals that regularly self-administer ethanol are more sensitive to salient stimuli, such as handling and transfer into the chamber, in general.

A number of studies have previously reported an increase in accumbal dopamine in trained rats after the presentation of cues that predict future ethanol availability, such as transfer of the animal into the operant chamber or an illuminated light inside the chamber, as well as the cues experienced during ethanol consumption (Doyon et al., 2005; Doyon et al., 2003; Gonzales and Weiss, 1998; Melendez et al., 2002; Nurmi et al., 1998). A previous study from our lab reported significant dopamine responses in the accumbens during transfer into the operant chamber and during consumption that were similar to those observed in the core-shell border in the present study (Doyon et al., 2005). The authors attributed the increase during consumption to stimuli associated with the drinking solution because of a dissociation between the ethanol and dopamine time-courses. The finding that dopamine

responds to ethanol predictive cues is consistent with numerous studies reporting that repeated exposures to a reinforcer, such as food reward or drugs of abuse, produce a temporal shift of both activation of dopamine neurons and increases in dopamine transients (Nishino et al., 1987; Roitman et al., 2004; Schultz et al., 1997; Stuber et al., 2008). The present finding of an increase in dopamine in the core-shell border during transfer into the chamber confirms these previous studies, and reveals for the first time that the response during consumption is restricted to this subregion.

The temporal shift of dopamine neuron activation from the time of the reinforcer to the time of the predictive cue is consistent with an idea put forth by numerous studies that mesolimbic dopamine, specifically in the accumbens, is more important for the appetitive phase than the consummatory phase of motivated behaviors (Blackburn et al., 1989; Ikemoto and Panksepp, 1996; Salamone et al., 1991). For example, disruption of dopamine signaling has been shown to affect responding for, but not consumption of, ethanol (Czachowski et al., 2002). However, these studies did not distinguish the accumbens core, core-shell border, and shell from one another. While the data from the core and shell in the present study confirm previous reports showing that dopamine in the accumbens is more important for the appetitive phase than the consummatory phase, the data from the core-shell border indicates that there is regional specificity to dopamine's role during the consumption phase of ethanol administration.

Although cues are likely to play a role in the dopamine response observed during transfer into the operant chamber, other factors may also contribute. For example, handling control groups that did not receive operant training in previous studies exhibited dopamine increases (20–30% above baseline) during transfer into the chamber, similar to those observed in groups drinking sucrose or water (Doyon et al., 2005; Doyon et al., 2003). The dopamine response in handling control groups could be caused by physical handling, change in environment, or a combination of the two, and indicates that much of the dopamine increase during transfer of animals trained to drink ethanol into the operant chamber may not be due to cues associated with the liquid reinforcer.

The reasons behind the larger accumbal dopamine response when first transferred into the operant chamber in 10S10E drinkers, when compared to 10S drinkers, are not known. However, it has been shown that dopamine responses to natural rewards, such as food, rapidly habituate, while those responses to drugs of abuse are persistent in nature (Bassareo and Di Chiara, 1999b; Di Chiara, 2002). It has also been suggested that dopamine responses mediate the formation of associations between cues and reinforcers (Di Chiara, 2002). Therefore, it is possible that the larger response in the ethanol drinkers reflects the formation of stronger associations formed between cues and ethanol, particularly compared to sucrose consumption.

Peak ethanol concentrations observed in the present study lead to levels of intoxication often observed in humans. The concentrations reaching the core and shell are not significantly different, with peak tissue levels achieved estimated to be 11.4 and 14.2 mM in the core and shell respectively (Howard et al., 2008). The peak core-shell border ethanol level is estimated to be 15.6 mM, and is very similar to shell levels. These levels would lead to mild intoxication in non-tolerant humans (equivalent to a blood alcohol concentration of 0.5 – 0.7 mg/ml), and would be achieved after consumption of approximately 2 – 3 standard drinks within 60 min (Brasser et al., 2004; Duarte et al., 2008; Erickson, 2007; Schweizer et al., 2006).

The 10S10E group differed from the 10S group in their drinking pattern in several ways that agree with a past study from our laboratory (Doyon et al., 2005). While the 10S10E group

drank mostly in the first five minutes, the 10S animals continued drinking into the second five minutes. The total number of licks, number of licks in first bout, duration of first bout, and milliliters consumed were all significantly lower in the 10S10E groups, which could be due to the intoxicating nature or the aversive taste and smell of the ethanol solution. These parameters were also lower for the 10S10E group of animals with probes sampling from the core-shell border. It is also important to note that we did not observe any differences between core, core-shell border, and shell groups for any of these lickometer parameters, indicating that probe placements did not influence these behaviors. Also, dopamine levels in the core and shell were not affected by these consumption behaviors as an increase in dopamine was not observed in either subregion during consumption of either of these drinking solutions.

In conclusion, the results of this study indicate that dopamine responses to ethanol self-administration are specific to accumbal subregions. Dopamine levels increase in a similar manner in the core and shell subregions, with increases occurring during the transfer of the rat to the operant chamber but not during drinking. However, dopamine in the core-shell border responds to transfer of the rat as well as during drinking. The results of this study also indicate that dopamine increases during transfer to the chamber are greater in animals expecting ethanol. Overall, these findings provide new details of accumbal dopamine function after the first week of ethanol self-administration.

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Figure 1.

Dialysate dopamine from the core and shell of the nucleus accumbens during transfer from home-cage into the operant chamber. * indicates a significantly larger dopamine response for the 10S10E trained rats (left) compared with the 10S trained rats (right). For rats drinking 10S10E, basal dopamine concentrations were 1.6 ± 0.2 nM for core ($n = 9$), and 1.5 ± 0.3 nM for shell ($n = 7$), and for rats drinking 10S, basal dopamine concentrations are 1.6 ± 0.2 nM for core ($n = 10$) and 1.4 ± 0.2 nM for shell ($n = 8$). Arrows indicate time of transfer of rat from home-cage into operant chamber. Mean $\pm$ sem are shown.

Figure 2.

Dialysate dopamine from the core and shell of the nucleus accumbens during the wait, drink, and post-drink periods. For rats drinking 10S10E (left), basal dopamine concentrations were 1.6 ± 0.2 nM for core ($n = 9$), and 1.5 ± 0.3 nM for shell ($n = 7$), and for rats drinking 10S (right), basal dopamine concentrations are 1.6 ± 0.2 nM for core ($n = 10$) and 1.4 ± 0.2 nM for the shell ($n = 8$). Arrows indicate when the bottle was lowered into the operant chamber. Mean $\pm$ sem are shown.

Figure 3. Dialysate ethanol from the nucleus accumbens core and shell during drinking and post-drinking periods. Dialysate ethanol concentrations are indicated (mean $\pm$ sem). Arrow indicates presentation of 10S10E drinking solution.

Figure 4.

Percentage of total licks during drinking period in 5 minute increments for core and shell animals drinking 10S or 10S10E. The open bars represent 10S10E drinkers and the filled bars represent 10S drinkers. * indicates a significant difference in percentage of licks for the 10S10E groups than the 10S group. Mean $\pm$ sem are shown.

Figure 5.

Dialysate dopamine from the core-shell border of the nucleus accumbens during transfer from home-cage into the operant chamber. * indicates a significantly larger dopamine response when compared to the wait period (collapsed across 10S10E and 10S). Basal dopamine concentrations were 1.1 ± 0.2 nM for rats drinking 10S10E ($n = 7$), and 1.2 ± 0.2 nM for rats drinking 10S ($n = 6$). Arrows indicate time of transfer of rat from home-cage into operant chamber. Mean $\pm$ sem are shown.

Figure 6.

Dialysate dopamine from the core-shell border of the nucleus accumbens during wait, drink, and post-drink periods. * indicates a significantly larger dopamine response for the 10S10E trained rats when compared to the wait period. # indicates a significantly larger dopamine response for the 10S trained rats when compared to the wait period. Basal dopamine concentrations were 1.1 ± 0.2 nM for rats drinking 10S10E ($n = 7$), and 1.2 ± 0.2 nM for rats drinking 10S ($n = 6$). Arrows indicate time of transfer of rat from home-cage into operant chamber. Mean $\pm$ sem are shown.

Figure 7.

Dialysate ethanol from the nucleus accumbens core-shell border during drinking and post-drinking periods. Dialysate ethanol concentrations are indicated (mean $\pm$ sem). Arrow indicates presentation of 10S10E drinking solution.

Figure 8.

Coronal sections indicating microdialysis probe placements in the core (light grey), core-shell border (yellow), and shell (not shaded) of the nucleus accumbens for the 10S ($n = 10$ for the core, $n = 6$ for the core-shell border, $n = 8$ for the shell) and 10S10E drinking groups ($n = 9$ for the core, $n = 7$ for the core-shell border, $n = 7$ for the shell). Core probes are shown in blue, core-shell border probes are shown in red, and shell probes are shown in dark grey. Numbers above slices denote the location in millimeters from bregma. This figure was adapted from Paxinos et al. (1999).

Table 1

Operant training procedure for drinking solution self-administration.

Day	Drink solution [^]	Pre-drink wait (min)	Response requirement
1	10S	2	2
2	10S2E	4	2
3	10S2E	6	2
4	10S5E	8	2
5	10S5E	10	4
6	10S10E	12	4
7	10S10E	15	4
8*	10S10E	15	4

The sucrose control group followed the same schedule except that ethanol was not faded into the drink solution.

[^] S stands for sucrose and E stands for ethanol. Numeral represents percentage (w/v for sucrose; w/v for ethanol).

* Dialysis session for all groups.

Lickometer parameters for rats with microdialysis probe in the core, shell, or core-shell border while self-administering ethanol plus sucrose (10S10E) or sucrose (10S).

Table 2

Parameter ^b	Core		Shell		Core-Shell Border	
	10S10E n=9	10S n=10	10S10E n=7	10S n=8	10S10E n=7	10S n=6
lever-pressing time (min)	0.08 ± 0.02	0.27 ± 0.15	0.09 ± 0.02	0.33 ± 0.25	0.21 ± 0.06	0.24 ± 0.11
latency to begin drinking (min)	0.11 ± 0.03	0.22 ± 0.10	0.10 ± 0.03	0.05 ± 0.01	0.17 ± 0.06	0.06 ± 0.02
number of bouts	1.2 ± 0.2	1.4 ± 0.2	1.4 ± 0.3	1.4 ± 0.2	1.3 ± 0.2	1.6 ± 0.2
initial bout duration (min)	5.2 ± 0.7	8.9 ± 1.3 ^a	5.9 ± 0.7	10.8 ± 1.0 ^a	7.2 ± 1.1	8.1 ± 1.6 ^a
total licks	1439 ± 129	2124 ± 219 ^a	1699 ± 198	2595 ± 172 ^a	1744 ± 271	2202 ± 211 ^a
Licks during initial bout	1343 ± 172	2005 ± 264 ^a	1645 ± 174	2481 ± 147 ^a	1652 ± 222	2071 ± 260 ^a
initial bout response rate (licks/min)	277 ± 22	245 ± 31	283 ± 23	243 ± 25	235 ± 17	276 ± 28
milliliters consumed	8.2 ± 0.9	10.9 ± 1.3 ^a	9.4 ± 1.0	12.2 ± 0.7 ^a	9.9 ± 1.4	12.3 ± 0.8 ^a

Values shown as mean ± sem

^aSignificantly different from the ethanol group (core, shell, and core-shell border combined) by multivariate ANOVA ($p < 0.05$)

^bParameters are defined as:

lever pressing time: time needed to complete response requirement (RR)
 latency to begin drinking: time between completion of RR and first spout lick
 bouts: period of at least 25 licks with no more than 2 min between licks
 initial bout duration: time needed to complete initial bout licks
 total licks: number of licks per session
 initial bout licks: number of licks during initial bout
 initial bout response rate: initial bout licks divided by initial bout duration
 milliliters consumed: volume consumed during session