Published in final edited form as:

Neuroscience. 2011 March 10; 176: 296–307. doi:10.1016/j.neuroscience.2010.12.016.

Fast dopamine release events in the nucleus accumbens of early adolescent rats

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Abstract

Subsecond fluctuations in dopamine (dopamine transients) in the nucleus accumbens are often time-locked to rewards and cues and provide an important learning signal during reward processing. As the mesolimbic dopamine system undergoes dynamic changes during adolescence in the rat, it is possible that dopamine transients encode reward and stimulus presentations differently in adolescents. However, to date no measurements of dopamine transients in awake adolescents have been made. Thus, we used fast scan cyclic voltammetry to measure dopamine transients in the nucleus accumbens core of male rats (29 – 30 days of age) at baseline and with the presentation of various stimuli that have been shown to trigger dopamine release in adult rats. We found that dopamine transients were detectable in adolescent rats and occurred at a baseline rate similar to adult rats (71 – 72 days of age). However, unlike adults, adolescent rats did not reliably exhibit dopamine transients at the unexpected presentation of visual, audible and odorous stimuli. In contrast, brief interaction with another rat increased dopamine transients in both adolescent and adult rats. While this effect habituated in adults at a second interaction, it persisted in the adolescents. These data are the first demonstration of dopamine transients in adolescent rats and reveal an important divergence from adults in the occurrence of these transients that may result in differential learning about rewards.

Keywords

Adolescence; dopamine; novelty; social interaction; voltammetry; nucleus accumbens

Introduction

Burst firing of dopamine neurons and the resulting dopamine release events (aka dopamine transients) are thought to comprise a key learning signal in the brain (Schultz, 2007; Roesch et al., 2010), interfacing external rewards and cues with appetitive behaviors. Dopamine transients occur spontaneously in several dopamine target regions (Robinson et al., 2002) and are more prominent at the presentation of unexpected stimuli (Rebec et al., 1997; Robinson and Wightman, 2004), social interaction (Robinson et al., 2001; Robinson et al., 2002) and rewards (Roitman et al., 2008). The fast dopamine release events are often followed by appetitive behaviors, such as approaching another rat or pressing a lever for a

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reinforcer (Robinson et al., 2002; Phillips et al., 2003; Roitman et al., 2004). Moreover, neutral cues that normally do not evoke dopamine transients can do so when repeatedly paired with a reward (Stuber et al., 2005b; Stuber et al., 2005a; Day et al., 2007), showing that this neural signal undergoes learning-induced plasticity. Importantly, dopamine transients are a result of volume transmission and achieve high extrasynaptic concentrations that can activate low-affinity, extrasynaptic dopamine receptors (Wightman and Robinson, 2002). Thus, dopamine transients appear to function as a brain signal of potential and established reward that can direct attention and facilitate the acquisition of that reward.

The mesolimbic dopamine system undergoes dynamic changes during adolescence in the rat. For example, expression of dopamine D1 and D2 receptors in ventral striatum increases from pre-adolescence to adolescence (e.g., Andersen et al., 1997), with some studies suggesting that binding in adolescence is higher than in adulthood (for references and discussion, see Doremus-Fitzwater et al., 2010; Wahlstrom et al., 2010b). Moreover, firing rates of dopamine neurons (McCutcheon and Marinelli, 2009) and basal dopamine concentrations (Badanich et al., 2006; Philpot et al., 2009) show similar U-shaped curves, peaking at adolescence. While dopamine transients have not yet been measured in adolescent rats, enhanced behavioral responses to novelty (Douglas et al., 2003; Stansfield and Kirstein, 2006; Philpot and Wecker, 2008) and social peers (Varlinskaya and Spear, 2008) have been reported in adolescent versus adult rats.

The present study aimed to provide the first measurements of dopamine transients in the nucleus accumbens (NAc) of adolescent rats and compare them to those in adults. We used fast scan cyclic voltammetry, an electrochemical technique with the spatial and temporal resolution required to detect dopamine transients (Robinson et al., 2008). We examined dopamine release in early adolescence (29 – 30 days of age) given that rats exhibit enhanced levels of peer-directed social interactions at this age relative to later adolescent and adult ages (Varlinskaya and Spear, 2008). Accordingly, we measured dopamine transients during brief social interactions as well as at baseline and at the presentation of unexpected, novel stimuli that have been reported to trigger dopamine transients in adult rats (Robinson and Wightman, 2004).

Experimental procedures

Animal subjects

All experiments described herein were approved by the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill, in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996). Male Long Evans rats were purchased from Charles River Laboratories (Raleigh, NC) in cohorts of four at postnatal day (PND) 21. Each rat was individually housed upon arrival with food and water *ad libitum*. Two of the rats from each cohort were assigned to the adolescent group and underwent surgery on PND 26 (67 ± 2 g) and voltammetric measurements on PND 29 or 30 (76 ± 3 g). The remaining two rats from each cohort were assigned to the adult group and underwent surgery on PND 68 (379 ± 12 g) and voltammetric measurements on PND 71 or 72 (379 ± 14 g); these rats were pair housed from PND 30 – 63.

Surgical preparation

The surgical procedures are as described previously (Robinson et al., 2009) with the following exceptions. Rats were anesthetized with isoflurane; adolescent rats were induced at 3% and maintained at 1 – 1.5% during surgery, while adult rats were induced at 5% and maintained at 2%. Rats were secured in a stereotaxic frame for implantation of a guide

cannula above the NAc, a bipolar stimulating electrode in the ventral tegmental area, and an Ag/AgCl reference, as previously described. The coordinates (versus bregma in mm) for the guide cannula were 1.3 anterior and 1.6 lateral (adolescent) or 1.7 anterior and 1.7 lateral (adult). The stimulating electrode was implanted at a 6° angle at the coordinates (versus bregma in mm) 4.1 posterior, 1.3 lateral (adolescent) or 5.2 posterior, 1.2 lateral (adult). Postoperatively, rats were closely monitored and received ibuprofen (15 mg/kg daily, p.o.) and palatable food.

Experimental design

Dopamine release was measured by using fast scan cyclic voltammetry as previously described (Robinson et al., 2009). Voltammetric recordings were made in a custom built Plexiglas chamber. Floor space was 21 × 21 cm with an angled insert that extended from the floor to 10 cm against the wall at a 48° angle; this prevented the rats from hitting the electrode and headstage assembly against the wall of the chamber. Rats were manually restrained while the carbon-fiber was lowered into the NAc core via the guide cannula, and then left undisturbed for 15 – 20 min. Next, voltammetric recordings were made every 2 – 4 min to assess the presence of naturally-occurring dopamine transients and electrically evoked dopamine release (16 – 24 pulses, 40 – 60 Hz, 120 μ A, 2 msec/phase, biphasic). Once we verified that the electrode was positioned near dopamine terminals, the experiment began; this was typically 60 – 85 min after initial placement in the chamber. Recording was continuous for 50 min. During the first 25 min, 5 stimuli were presented for 3 s at 5-min intervals in a random order: house light off, tone, white noise, coconut odor and lemon odor. The odors were presented in the following manner (Robinson and Wightman, 2004): the experimenter dipped a cotton applicator into an extract (McCormick), opened the recording chamber door, held the applicator 1 – 2 cm from the rat's nose for 3 s, then withdrew and closed the chamber door. After presentation of all stimuli, recording continued for 5 min, then another male Long Evans rat was placed in the cage with the test rat for 60 s and allowed to interact. For the adolescent group, the conspecific rat was age-matched to the test rat; for the adults, the partner was a slightly smaller adult ($87 \pm 6\%$ of test rat weight) in an attempt to prevent the emergence of aggressive interactions. A second 60-s interaction period occurred with the same partner 10 min later. After recording, rats were given a lethal dose of urethane (> 1.5 g/kg, i.p.) then perfused with a formalin solution. The brains were removed, frozen, sectioned (40- μ m thickness) and stained with thionin to determine recording sites.

Evaluation of dopamine transients

Dopamine transients during recording were statistically identified as previously described (Robinson et al., 2009). In brief, each voltammetric scan was background-subtracted and compared to a template of electrically-evoked dopamine release. After identification of dopamine release events, each event was examined to determine the signal-to-noise ratio, with the signal being the maximal concentration of dopamine ($[DA]_{\max}$) and the noise calculated as the rms amplitude in the 10 scans (1 s) used in the background subtraction. Only dopamine transients with a signal-to-noise ratio of 5 or higher were included in the study. To determine dopamine release during stimulus presentations, the frequency of transients was calculated for a 20-s period encompassing the stimulus presentation and compared with the frequency during the 4 min immediately preceding the presentation (basal). To determine dopamine release during social interaction, the frequency of transients was calculated for an 80-s period encompassing the interaction period and compared with the frequency during the 4 min immediately preceding the interaction (basal). The maximal concentration of each dopamine release event was calculated by converting the current (nA) to concentration (μ M) after *in vitro* calibration of the electrode (Logman et al., 2000).

Behavioral scoring

Behavioral response to stimuli presentations (from initiation of presentation to 2 s following the presentation) was rated from video on a scale of 0 – 3 (0, no movement; 1, sniffing/head movement; 2, orientation movement/startle; 3, locomotion). Social interaction was scored as the overall duration in s of the following behaviors directed toward the other rat: grooming, sniffing, pursuing, ano-genital inspecting, climbing over/under. Trials were scored for social behaviors occurring in the 45 s immediately following closure of the sound-attenuating chamber door after inserting the partner rat into the chamber; this time span was chosen to avoid any confounding influence of opening and closing the chamber doors. Trials were also scored for duration in s of non-social locomotor activity via quadrant crossings during the same 45-s period.

Statistical analysis

Statistical analysis of dopamine transient frequency between groups and experimental conditions was calculated by using nonparametric multivariate regression analysis (genmod procedure with Poisson distribution, repeated measures and Wald pairwise contrasts; SAS, SAS Institute Inc., Research Triangle Park, NC). Changes in amplitude of dopamine transients and signal-to-noise ratios between groups and experimental epochs were calculated using similar regression analysis with gamma distribution. Behavioral measures were compared between groups by using repeated-measures 2-way ANOVA or within groups by using paired t-tests (GraphPad Prism, GraphPad Software Inc., La Jolla, CA).

Results

Twelve adolescent and ten adult rats were used for this study. Three adolescent rats were not included due to difficulty at surgery or during voltammetry, and one adult rat was not included due to an incorrect electrode placement. In addition, social interaction data from one adolescent was discarded due to voltammetric difficulties that did not affect the other stimulus presentations. Final n's were 9 adolescents and 9 adults for the stimulus presentations and 8 adolescents and 9 adults for the social interactions.

Dopamine transients at baseline

Basal rates of dopamine transients were overlapping between adolescent and adult rats in the NAc core. When transient frequency was determined across all baseline samples (files in which no stimulus presentations or rat interactions occurred), adolescent rats exhibited 1.5 ± 0.4 transients/min and adults exhibited 2.5 ± 0.6 . However, within each group was a range of transient frequency, as previously reported in mature rats (Robinson et al., 2009; Robinson and Wightman, 2007; Wightman et al., 2007): in adolescents, basal frequency ranged from 0.2 – 4.0 transients per min, while in adults the range was 0 – 5.0 transients per min. Importantly, electrically-stimulated release revealed the presence of functional dopamine terminals at the voltammetric recording site even when basal dopamine transients were absent or infrequent.

Dopamine transients at the presentation of unexpected stimuli

Next, we investigated whether dopamine transients were more likely to occur at the unexpected presentation of stimuli in adolescent rats. Figure 1A shows that brief presentations of stimuli increased the frequency of dopamine transients by 50% above baseline levels in adult rats (Wald contrast of adult baseline vs. stimuli, $p < 0.05$), replicating previous work (Robinson and Wightman, 2004). In contrast, the rate of dopamine release events did not change significantly among the adolescent rats (Wald contrast of adolescent baseline vs. stimuli, $p > 0.86$). When compared between groups, baseline rates did not differ

(Wald contrast of adolescent vs. adult baseline, $p > 0.19$), while rates during stimulus presentations were higher in adult rats (Wald contrast of adolescent vs. adult stimuli, $p < 0.05$). Adult rats were more likely to have dopamine release time-locked to the stimuli: 8/9 adults exhibited dopamine release to at least one stimulus presentation, while 6/9 adolescents did so. Moreover, adult rats that exhibited time-locked dopamine transients did so at 3.8 ± 0.4 stimuli (median 4), while adolescents that exhibited time-locked transients did so at 2.3 ± 0.4 stimuli (median 2).

We next determined whether transients occurring during stimulus presentations were larger than those during baseline. Figure 1B shows the distribution of the maximal concentrations of dopamine transients. Regression analysis revealed that amplitudes did not differ between adolescent and adult rats (Wald contrasts, all p values > 0.05). In adolescent rats, the maximal concentrations of transients during stimuli presentations were not significantly different from those during baseline. In adult rats, dopamine concentrations were slightly higher during stimulus presentations, a rightward shift that approached statistical significance (Wald contrast of adult baseline vs. stimuli, $p < 0.06$). The median and average concentrations of dopamine transients are described in Table 1.

Although group data indicated that dopamine release events were more likely to occur at unexpected stimulus presentations in adults versus adolescent rats, individual plots illustrate the differences among stimuli as well as within groups. Figure 1C shows the change in frequency of dopamine transients during the presentation of a stimulus versus the 4-min of baseline preceding that stimulus. In adults, the stimuli varied in their effectiveness to increase dopamine transient rates; they are ranked from most to least effective as follows: dopamine transients occurred at 208% of basal rates at coconut odor; 184% at tone; 161% at lemon odor; 142% at white noise; and 91% at light out. In adolescent rats, stimuli were overall less effective to trigger dopamine transients; they are ranked from most to least effective as follows: dopamine transients occurred at 158% of basal rates at the tone; 127% at lemon odor; 123% at coconut odor; 84% at white noise; and 23% at light out.

Nevertheless, Figure 1C demonstrates distinct individual variability in emitting a dopamine response to particular stimuli within each age group. To determine whether this neurochemical variability was associated with behavioral variability, we scored each rat for movement associated with the stimulus presentation, as shown in Table 2 (one adult rat was excluded from this analysis due to partial loss of the video record). We then used the Spearman nonparametric correlation to compare behavioral and dopaminergic responses to stimuli within each age group. We found that the frequency of dopamine transients was not correlated with behavioral activation in either age group at any stimulus, regardless of whether the stimulus was analyzed individually or as a group (data not shown, all p values > 0.05), indicating that the behavioral and neurochemical responses to stimuli were not directly related. In addition, the behavior scores of the rats that did not emit any dopamine transients time-locked to stimulus presentations (3/9 adolescents and 1/9 adults) completely overlapped with the behavior scores of the rats that did exhibit dopamine transients. However, comparison of behavioral responses between age groups revealed that adolescents as a group displayed significantly less movement at unexpected stimulus presentations than the adult rats (Mann-Whitney test, $p < 0.05$).

Dopamine transients during social interaction

Next we measured dopamine release during 60-s interaction with another male rat. In contrast to the presentations of the non-social stimuli, regression analysis determined that dopamine transients significantly increased from baseline during the first interaction with another rat in both adolescent and adult rats. The average rate of transients increased 3-fold from baseline, from 1.0 ± 0.3 to 3.0 ± 0.9 transients/min in adolescent rats (Wald contrast of

adolescent baseline vs. interaction, $p < 0.05$) and from 2.0 ± 0.5 to 7.3 ± 1.3 in adults (Wald contrast of adult baseline vs. interaction, $p < 0.001$). Ten min later, dopamine release was measured during a second interaction with the same rat. In adults, the increase in dopamine transient rate was no longer significant ($p > 0.32$); although the average rate of transients increased from 1.8 ± 0.5 to 3.7 ± 1.3 transients/min, this change was more variable across rats. In contrast, adolescents exhibited the same increase in dopamine transients during the second presentation as during the first, from 0.8 ± 0.2 to 3.1 ± 0.9 transients/min ($p < 0.01$). Individual rat data by group and episode are shown in Figure 2A.

We investigated whether differences in behavior during the first and second interaction episode might explain the lack of increase in dopamine release in adult rats during the second interaction. Table 3 shows the time spent in social investigation and locomotion during the 45 s of scored interaction period. In this experimental chamber (21×21 cm floorspace), adolescent rats spent less time in active social interactions (2-way repeated-measures ANOVA: main effect of group, $p < 0.05$) and more time in locomotion (2-way repeated-measures ANOVA: main effect of group, $p < 0.05$) than did adult rats. However, we observed that the adolescent rats had more space to move apart from each other while the larger adult rats were more likely to be in physical proximity. Thus, the critical comparisons were paired t-tests to compare within-group behavior during the first versus second interaction periods. These analyses revealed no behavioral differences between the first and second interaction periods in either age group (all p values > 0.05). Moreover, neither the absolute frequency of dopamine transients nor the increased frequency from baseline correlated with social interactions or locomotion during the interaction period (data not shown, all p values > 0.05).

The maximal concentration of dopamine transients during interaction with another rat was compared to concentrations during baseline. For this analysis, data from both interaction episodes were pooled to increase statistical power. Figure 2B shows that the distribution of dopamine transient amplitude was shifted toward larger release events in both adolescent and adult rats during conspecific interaction; average and median amplitudes are listed in Table 1. Because social interactions can produce movement-associated noise in the voltammetric signal, we also investigated noise levels and found that they were indeed higher during interaction episodes in both adult and adolescent rats (Wald contrasts of baseline vs. interaction, $p < 0.05$ for each group). Nevertheless, the signal-to-noise ratios did not differ across groups (Table 1: Wald contrasts, all p values > 0.05), suggesting that noise issues did not contribute to the differential findings between adolescent and adult rats.

All recordings were made in the core of the NAc, as shown in Figure 3.

Discussion

Dopamine neurotransmission is key to many aspects of motivated behavior, including stimulus salience, reward prediction and behavioral facilitation. As motivated behavior differs between adolescents and adults, the present study surveyed fast dopamine release events in early adolescent rats in comparison to adults. We report that while basal rates of dopamine transients do not differ significantly between the two age groups, transients in response to unexpected stimuli are fewer in adolescent rats as compared to adults. In contrast, both frequency and amplitude of dopamine transients increase in both age groups at interaction with another rat; however, the change in frequency habituates in adults but not adolescents at the second presentation of the partner rat. Thus, fast dopamine release events at the presentation of stimuli differ in adolescent rats versus adults, and this physiological difference may be associated with age-dependent differences in the processing of social and nonsocial stimuli.

Both frequencies and amplitudes of dopamine transients in the NAc core were similar between male rats in early adolescence (age 29 – 30 days) and adulthood (age 71 – 72 days). These findings are consistent with electrophysiological recordings of dopamine neurons in anesthetized rats. Firing rates of dopamine neurons increase from birth to mid-late adolescence, then decline across adulthood (Pitts et al., 1990; Tepper et al., 1990; Lavin and Drucker-Colin, 1991; Marinelli et al., 2006; McCutcheon and Marinelli, 2009), with peak activity occurring in mid to late adolescence. Indeed, McCutcheon and Marinelli (2009) reported that basal firing rates are similar at the early adolescent and adult ages targeted in the present study, and the data herein suggest that rates of neuronal bursting are also similar, as dopamine transients arise from burst firing of dopamine neurons (Suaud-Chagny et al., 1992; Sombers et al., 2009). Notably, adolescent rats exhibited variability in the basal frequency of dopamine transients, ranging from sites with few to no spontaneous transients to sites with several per minute. This finding is similar to “hot” and “cold” recording sites reported in adults (Robinson et al., 2009; Robinson and Wightman, 2007; Wightman et al., 2007) and may reflect variability in burst rates of dopamine neurons (Hyland et al., 2002). Consistent with developmental firing rates, microdialysis studies have reported increased dopamine levels in later adolescence (45 days of age) as compared to early adolescence or adulthood (Badanich et al., 2006; Philpot et al., 2009). Thus, while our findings provide the first evaluation of basal rates of dopamine transients between adolescent and adult rats, more timepoints are needed during this dynamic developmental period as dopamine transients might be more prominent during mid-late adolescence.

Consistent with our previous report (Robinson and Wightman, 2004), we found that the frequency of dopamine transients increased at the unexpected presentation of stimuli in adult male rats, often time-locked to the initial presentation of the stimulus. In the former study, we presented odor and auditory stimuli similar to those used herein; in both studies, these stimuli increased the frequency of dopamine transients in the NAc above basal rates, which was interpreted as a neurochemical signal of potential salience of the stimuli to the animal. However, the increase in dopamine transient frequency in response to unexpected presentation of stimuli observed in adults was not reliably present in the NAc core of early adolescent rats, and the adolescents as a group displayed less behavioral response to the stimuli than did adults. Although phasic activation of dopaminergic neurons is not correlated with specific motor movements, the types of stimuli that tend to promote burst firing and dopamine transients often induce behavioral activation (Nishino et al., 1987; Romo and Schultz, 1990; Robinson et al., 2002). Thus, the present findings indicate a developmental difference in the dopaminergic and behavioral response to this type of novel stimulus presentation, which may both be due to a lack of salience of these stimuli for the adolescent rats. Importantly, the reduced behavioral response to stimuli was not simply a reduction in the ability to move (perhaps due to the tether or the voltammetric headstage), as the adolescent rats exhibited both locomotion and socially directed behaviors during social interaction. Moreover, there was no association between behavioral response and the frequency of dopamine transients to stimuli when analyzed across individual rats within each age group. The relative insensitivity to unexpected, novel stimuli found in the present study contrasts with prior findings that adolescent rats often exhibit higher levels of exploration of novel environments and novel objects than adults (e.g., Douglas et al., 2003; Stansfield and Kirstein, 2006; Philpot and Wecker, 2008), with novel object exploration reported to peak in rats in mid-adolescence (35 – 36 days of age, Spear et al., 1980). Additional studies would be necessary to determine ontogenetic differences between behavioral activation and concurrent dopamine release events induced by the brief stimulus presentations used herein versus responses to novel environments and static, novel objects placed into familiar environments as used in the previous studies.

Both the frequency and amplitude of dopamine transients in the NAc core reliably increased during brief interaction with another rat in both adolescent and adult groups. Consistent with our previous report (Robinson et al., 2002), the dopaminergic response habituated in adult rats at the second presentation of the conspecific rat. In contrast, the increased frequency of dopamine transients persisted in adolescent rats. This lack of habituation may reflect the increased reward associated with social interaction exhibited in adolescent versus adult rats (e.g., Douglas et al, 2004). Indeed, social activity has been shown to peak in early adolescence compared to later adolescence and adulthood, an effect that is magnified when rats are isolate-housed for the days before the testing (Varlinskaya & Spear, 2008), as was done in the present study. Interestingly, the two age groups differed on the overall amount of social activity versus general locomotion. While previous reports indicate that isolate-housed adolescent rats exhibit more social behavior and locomotion than adults during discrete social interaction trials, with these effects being particularly pronounced among early adolescents (Varlinskaya & Spear, 2008), we found that locomotion was higher and social-directed behavior was lower in the early adolescents we tested when compared with their adult counterparts. This may be due to the size of the apparatus: at 21×21 cm, the larger adult rats were more likely to be in close proximity to the conspecific partner than the smaller adolescent rats, making social contact almost inevitable for these adult animals. In addition, having only the test rat tethered may have affected its behavioral repertoire during social interaction. Finally, the short time period of interaction (60 s) used herein captures only initial interactions which may produce different age-related patterns of social behavior when compared to the longer interaction periods (270–600 s) typically used in terms of rodent social interactions (e.g., Varlinskaya & Spear, 2008; Glenn et al, 2003).

Social interaction can induce movement-associated electrical noise as the headstage assembly touches the chamber wall or the other rat which may lead to underestimation of dopamine transient frequency during social interaction. Importantly, signal-to noise levels were not different between age groups or between the first and second interaction periods, indicating that the persistent increase in dopamine transient frequency observed in adolescent rats during the second interaction period was not a noise-related artifact. Similarly, investigative and locomotor behavior was essentially the same during both interactions within each age group, so behavioral differences also do not explain the difference in habituation of the dopaminergic response between adolescent and adult rats. Indeed, in our previous study (Robinson et al., 2002), the observation was made that adult rats exhibited more intense socially directed behaviors during a second interaction with a partner rat despite emitting fewer dopamine transients, suggesting that the dopamine transients are not necessary to promote partner-directed behaviors. If the dopamine transients are interpreted as signals of reward prediction (Schultz and Dickinson, 2000; Schultz, 2007; Roesch et al., 2010), the habituation of dopamine release events in adults to repeated partner presentations may reflect a decrease in reward or greater predictability of the second presentation, and the persistence of dopamine release in the adolescent rats may reflect enhanced reward or surprise to repeated interaction with a partner.

The mesolimbic dopamine system is involved in appetitive behaviors and reward acquisition (for reviews, see Depue and Iacono, 1989; Panksepp, 1998; Depue and Collins, 1999; Ikemoto and Panksepp, 1999; Schultz and Dickinson, 2000; Schultz, 2007). As several aspects of this dopamine pathway undergo dynamic changes during adolescence, it is not surprising that behavioral and neurochemical responses to rewards and novel stimuli that may well predict rewards are also dynamic (for reviews, see Chambers et al., 2003; Ernst et al., 2009; Wahlstrom et al., 2010b; Wahlstrom et al., 2010a). Our finding that the dopaminergic response to social interaction did not habituate in early adolescent rats is consistent with many studies that have documented heightened sensitivity to rewards during adolescence, including social and drug reward (for review and references, see Doremus-

Fitzwater et al, 2010; Spear and Varlinskaya, 2010). Novel stimuli likewise are salient and may trigger dopamine release and behavioral facilitation because they could predict reward or threat; however, we did not observe increased dopamine release to brief presentation of novel stimuli in early adolescent rats. Thus, the present study may have sampled dopamine release at a time in development (early adolescence) during which social reward sensitivity is optimal but response to novelty is not. This interpretation leads to several avenues of further research, including examination of dopamine release to novel and social stimuli at more timepoints across adolescence. We also plan to investigate dopamine release during explicit cue-reward learning (e.g., Pavlovian conditioning) to determine whether the types of stimuli presentations used herein, such as a light or odor, can evoke a dopaminergic response in adolescent rats when they predict reward (Day et al, 2007; Roesch et al., 2007).

In summary, fast dopamine release events, or dopamine transients, are differentially expressed in early adolescence versus adulthood. While rates and concentrations of transients were similar at baseline, we observed less activation of dopamine release by unexpected, nonsocial stimuli and more persistent activation by social stimuli in adolescent rats as compared to adults. These differences in dopamine release events likely contribute to developmental differences in sensitivity to cues and rewards, particularly social reward. It will be valuable to build on these findings by evaluating more timepoints during adolescence as well as monitoring dopamine release during explicit reward-associated learning.

Acknowledgments

Thanks to Dr. Thomas Guillot III for assistance with neuroanatomical coordinates, to Rachel Hay and Sebastian Cerdana for behavioral scoring, to Vahid Sanii for electrode calibration, and to Chris Wiesen at the UNC Odum Institute for Research in Social Science for statistical expertise. This work was funded by NIH (R01DA019071 to L.P.S.) and the Bowles Center for Alcohol Studies at the University of North Carolina.

Abbreviations

[DA] _{max}	maximal concentration of dopamine
NAc	nucleus accumbens

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Figure 1.

Dopamine release events in adolescent and adult rats during the presentation of unexpected stimuli. (A) In adolescent rats dopamine transient frequency did not change from basal rates during presentation of stimuli. In contrast, the frequency of dopamine transients increased at stimulus presentations in adult rats (* $p < 0.05$ versus baseline). (B) The amplitude of dopamine transients during stimulus presentations did not significantly change from baseline in either age group. Bin size is 0.01 μM . (C) Frequency of dopamine transients in individual adolescent and adult rats before (“Basal”) and during presentations of stimuli (“Stim”). Fewer adolescent rats than adult rats exhibited increased dopamine transients to unexpected stimuli.

Figure 2.

Dopamine release events in adolescent and adult rats during brief interaction with a size-matched rat. (A) Dopamine transients were more frequent in both adolescent and adult rats during the first interaction with another rat (“Rat”) versus immediately before (“Basal”). However, only adolescents exhibited a second significant increase in dopamine transient rate during a second interaction 10 min later (bottom). (B) The distribution of dopamine transient amplitudes shifted to higher concentrations during conspecific interaction versus baseline in both age groups. Bin size is 0.01 μM .

Figure 3. Dopaminergic recording sites within the nucleus accumbens core of adolescent and adult rats, shown on representative coronal slices at 1.2 and 1.6 mm anterior to bregma (adapted from Paxinos and Watson, 1986).

Table 1

Maximal concentrations of dopamine release events in the nucleus accumbens core of adolescent and adult rats.

Group	Stimulus presentations		Interactions with another rat	
	Baseline	Stimuli	Baseline	Interaction
Adolescent				
[DA] _{max} , μ M, Mean $\pm$ S.E.	0.032 $\pm$ 0.001	0.032 $\pm$ 0.003	0.028 $\pm$ 0.001	0.049 $\pm$ 0.006 ^a
[DA] _{max} , μ M, Median	0.027	0.027	0.027	0.038
Signal-to-noise	8.9 $\pm$ 0.3	9.5 $\pm$ 1.0	8.6 $\pm$ 0.6	10.0 $\pm$ 1.2
Adult				
[DA] _{max} , μ M, Mean $\pm$ S.E.	0.034 $\pm$ 0.001	0.042 $\pm$ 0.003 ^b	0.033 $\pm$ 0.001	0.046 $\pm$ 0.002 ^c
[DA] _{max} , μ M, Median	0.030	0.036	0.028	0.042
Signal-to-noise	9.4 $\pm$ 0.3	10.1 $\pm$ 0.7	8.6 $\pm$ 0.5	9.5 $\pm$ 0.7

^a significantly higher than baseline, $p < 0.01$;

^b marginally higher than baseline, $p < 0.06$;

^c significantly higher than baseline, $p < 0.001$

Table 2

Behavioral scores for adolescent and adult rats at the presentation of unexpected stimuli (0 = no movement; 1 = sniffing/head movement; 2 = orientation movement/startle; 3 = locomotion).

Stimulus	Adolescent	Adult
Coconut	1.9 ± 0.3	3.0 ± 0.0
Lemon	1.9 ± 0.3	3.0 ± 0.0
Tone	0.6 ± 0.2	0.9 ± 0.1
White noise	1.7 ± 0.3	2.1 ± 0.3
Light out	0.6 ± 0.3	0.5 ± 0.4

Table 3

Time (s) spent in social investigation and locomotion during interaction with another rat.

Group	Social investigation ^a		Locomotion ^a	
	1 st interaction	2 nd interaction	1 st interaction	2 nd interaction
Adolescent	20.8 ± 4.6	19.1 ± 3.4	7.3 ± 2.0	6.5 ± 1.7
Adult	31.6 ± 3.8	35.6 ± 4.0	3.8 ± 0.9	2.3 ± 0.6

^a main effect of age, $p < 0.05$