Archival Report

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Reward Anticipation Is Encoded Differently by Adolescent Ventral Tegmental Area Neurons

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ABSTRACT

BACKGROUND: Elucidating the neurobiology of the adolescent brain is fundamental to our understanding of the etiology of psychiatric disorders such as schizophrenia and addiction, the symptoms of which often manifest during this developmental period. Dopamine neurons in the ventral tegmental area (VTA) are strongly implicated in adolescent behavioral and psychiatric vulnerabilities, but little is known about how adolescent VTA neurons encode information during motivated behavior.

METHODS: We recorded daily from VTA neurons in adolescent and adult rats during learning and maintenance of a cued, reward-motivated instrumental task and extinction from this task.

RESULTS: During performance of the same motivated behavior, identical events were encoded differently by adult and adolescent VTA neurons. Adolescent VTA neurons with dopamine-like characteristics lacked a reward anticipation signal and showed a smaller response to reward delivery compared with adults. After extinction, however, these neurons maintained a strong phasic response to cues formerly predictive of reward opportunity.

CONCLUSIONS: Anticipatory neuronal activity in the VTA supports preparatory attention and is implicated in error prediction signaling. Absence of this activity, combined with persistent representations of previously rewarded experiences, may provide a mechanism for rash decision making in adolescents.

Keywords: Adolescent, Dopamine, Extinction, Instrumental learning, Reward, Ventral tegmental area

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Adolescence is associated with risky behavior and the symptomatic onset of major psychiatric disorders (1–3). Immaturities in processing rewarding experiences have been suspected in adolescents and are thought to contribute to poor decision making and increased susceptibility to develop addictive and psychiatric disorders (1,4,5). The dopamine system has been implicated strongly in adolescent behavioral and illness vulnerabilities (6–8). In the adult brain, dopamine neurons in the ventral tegmental area (VTA) are critically involved in reward processing (9) and have been implicated in the pathophysiology of addiction, mood disorders, and schizophrenia (10,11). In the adolescent brain, there is a general assumption of exaggerated VTA or dopamine responses to reward (12–14). But while a few studies suggest differences in dopamine-related measures between adolescents and adults (6,15,16), little is known about how adolescent VTA neurons process reward-related events.

We recorded daily from populations of VTA neurons in adolescent (postnatal days [P] 38 to 45) and adult rats during learning, brief maintenance, and extinction of a cued reward-driven instrumental task. Animals first learned that a rewarding outcome would be available if they performed an instrumental action (nose poke) after cue presentation and then, during extinction, learned that the reward was no longer available after cue presentation. This task was chosen because it 1) is sensitive to dopamine neurotransmission; 2) is feasible to complete during the short adolescent period; 3) provides measures of reward-mediated learning and extinction, which are critical processes for adapting to changes in environmental association (17); and 4) is learned and performed similarly by adolescents and adults, allowing the confound of potential behavioral differences contributing to neuronal activity differences to be ruled out. Data were analyzed considering the response of all VTA neurons, as well as comparison between neurons with nondopamine or dopamine-like characteristics. These analyses revealed critical age-related differences in VTA neuronal activity, which may provide novel insight for understanding dopamine-related behavioral vulnerabilities in adolescents.

METHODS AND MATERIALS

Subjects and Electrode Implantation

Experimental procedures were in accordance with the University of Pittsburgh Institutional Animal Care and Use Committee. Male Sprague Dawley (Harlan, Frederick, Maryland) adolescent (n = 7) and adult (n = 11) rats were used in this experiment. Surgical methods are as described previously (18). Adolescents (P21) and adults (P70+) were received at least 1 week before surgery (approximately P28 in the adolescent group) to habituate to the vivarium (12-hour light/dark cycle with lights on at 7:00 PM). We implanted laboratory-made 8-channel electrode arrays (50-μm-diameter tungsten

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wire insulated with polyimide; California Fine Wire Company, Grover Beach, California) into the VTA of adults (anterior-posterior, −5.3; medial-lateral, .8; dorsal-ventral, 8.1) and adolescents (anterior-posterior, −4.9; medial-lateral, .7; dorsal-ventral, 7.5). Animals recovered 7 to 8 days from surgery. Daily recordings were performed in adolescents beginning on P38 and ending on P45.

Behavior

Animals were first habituated for 2 days to an operant chamber (Coulbourn Instruments, Allentown, Pennsylvania) equipped with a food trough and reward magazine opposite a nose-poke port with a cue light and infrared photodetector unit and a tone-generating speaker (Figure 1A). Similar to previous work (18), rats then learned to nose poke into the lit port for reward delivery in six sessions. Each trial began with the cue onset (the cue light and 500-Hz tone .2 second in duration). Following the nose poke (action), the cue light was extinguished and a reward was delivered 2 seconds later. A 15-second intertrial interval (ITI) initiated upon reward collection (45-mg sugar pellet; Bio-Serv, Frenchtown, New Jersey), after which began the next trial. For each trial, the cue light remained illuminated until the rat responded. Each session lasted 30 minutes. In session 7, actions were reinforced for 30 trials (session 7R). Following this, reward was no longer available during a 30-minute block of trials (within-session extinction [session 7E]). In session 8 (30 minutes), the cue light still was extinguished after an action, but no reward was delivered and the trial proceeded to the ITI (full session of extinction).

Electrophysiology

Rats were connected to a lightweight headstage (Plexon, Dallas, Texas) and a motorized commutator (Plexon) that allowed free movement during experiments. Data were high-pass filtered at 100 Hz and digitized at 40 kHz (OmniPlex System; Plexon). Data were sorted with standard techniques (Offline Sorter; Plexon); minimum acceptable signal-to-noise ratio approximately 3:1. Neurons were not prescreened for physiological characteristics or response properties before recording. Behavioral events were synchronized with neuronal activity. NeuroExplorer (NEX Technologies, Madison, Alabama) was used for preliminary analysis.

Data Analysis

The number of trials completed served as a behavioral index of learning and performance. A two-way repeated measures analysis of variance (ANOVA), with session as a repeated measure, was used to compare behavioral performance between age groups and sessions. Independent samples *t* tests were used to quantify age-related behavioral differences in single sessions. Greenhouse-Geisser corrections were applied in all cases in which unequal variances between groups were detected. Isolated single unit data were analyzed with custom written MATLAB functions (The MathWorks, Natick, Massachusetts). We classified neurons recorded in consecutive recording sessions as different units, despite the possibility that units were recorded serially. This approach is the most conservative assessment of unit identity. Though neuronal classification is necessarily arbitrary, for comparison with previous work, neurons were classified as dopamine-like or nondopamine-like based on baseline firing rate (dopamine-like neurons < 10 Hz) and width of waveform (dopamine-like neurons > 1.2 ms). Note that, because the adolescence period in rodents is short (~10 days), use of optogenetics to identify dopamine or gamma-aminobutyric acid (GABA) neurons is not feasible because there is not enough time after weaning to allow for sufficient virus expression after surgery.

Basal activity levels were measured the final 10 seconds of the ITI. Baseline firing rates in both age groups were non-normally distributed and compared with a Wilcoxon rank sum test. Unit firing rates during task events were binned (50 ms), smoothed with a five-point moving rectangular kernel, and *Z*-score normalized against a stable baseline period (−3.5 to −.5 second from cue onset). A .25-second window, beginning at cue onset, was utilized for cue-evoked response. The neuronal responses around instrumental action execution were analyzed in both small (.5 second) and large (.75 second) windows that were centered on the time of the action. This activity may be evoked directly by the action or

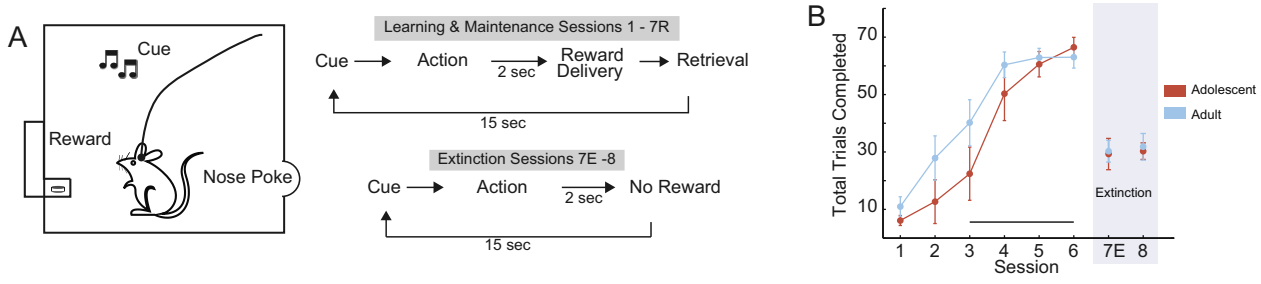

Figure 1. Adult and adolescent rats learn instrumental associations and extinction at similar rates. **(A)** Task schematic: When rats nose poked into the lit port, a reward was delivered following a 2-second delay. After animals retrieved the sugar reward, a 15-second intertrial interval began. Sessions 1 to 6 proceeded in this fashion for 30 minutes each (Learning & Maintenance). Session 7 began with 30 rewarded trials (7R) and then proceeded to 30 minutes of extinction (7E) in which responses were not rewarded (Extinction). Session 8 was 30 minutes of extinction trials only. **(B)** There were no differences between adolescents ($n = 7$) and adults ($n = 11$) in total trials completed per session. During extinction in sessions 7 and 8, there were no group differences in trials completed. Total trials completed in each session are depicted as the session mean ± SE, across all animals in an age group. Data are depicted for sessions 1 to 6 (before extinction) and sessions 7E and 8. Underlined sessions (sessions 3 to 6) and extinction sessions were used for electrophysiological analysis. The shaded portion of the figure depicts extinction sessions. Data from session 7R are not plotted, as all animals were required to complete exactly 30 trials.