Developmental changes in habenular and striatal social reinforcement responsivity across adolescence linked with substance use.

Jessica S. Flannery[1*], Nathan A. Jorgensen[1], Seh-Joo Kwon[1], Mitchell J. Prinstein[1], Eva H. Telzer[1], Kristen A. Lindquist[1]

[1]Department of Psychology & Neuroscience, University of North Carolina at Chapel Hill, Chapel Hill, NC

*__Correspondence:__
Dr. Jessica S. Flannery
jflannery@unc.edu
University of North Carolina, Chapel Hill
235 E. Cameron Avenue
Chapel Hill, NC 27599-3270

Abstract: 233 words
Text: 3,999 words
Tables: 1
Figures: 4
References: 71
Supplemental materials: 1 file

RUNNING TITLE: Habenula, development, and substance use

KEYWORDS: habenula, striatum, substance use, adolescence, dopamine, social reinforcement

ABSTRACT

Background: Habenula (HB) function is implicated in substance use disorders and is involved in inhibiting dopamine release in the ventral striatum (VS). While blunted VS reward-responsivity is implicated in risk for later substance use, links between HB reinforcement processing and progression of use has not been examined among adolescents. In the present study, we longitudinally assess HB and VS responsivity to social rewards and punishments, across adolescence, and examine associations with substance use.

Methods: Within a longitudinal design, 170 adolescents (53.5% female) completed 1-3 fMRI scans across 6^{th}-9^{th} grade and reported yearly substance use across 6^{th}-11^{th} grade. We examined VS and HB responsivity to social reinforcement during a social incentive delay task in which adolescents received social rewards (smiling faces) and punishments (scowling faces).

Results: We observed increased VS responsivity to social rewards (vs. reward omissions) and increased VS, but decreased HB, responsivity to social punishment avoidance vs. receipt. However, contrary to hypotheses, the HB displayed increased responsivity to social rewards (vs. reward omissions). Further, adolescents reporting regular substance use displayed longitudinally declining HB responsivity to social rewards (vs. reward omissions), whereas adolescents reporting no substance use displayed longitudinally increasing HB responsivity. In contrast, whereas VS responsivity to punishment avoidance vs. receipt increased longitudinally among regular substance users, it stayed relatively stable among non-users.

Conclusions: These results suggest differential HB and VS social reinforcement processing trajectories across adolescence are associated with substance use.

INTRODUCTION

Substance use often begins during adolescence (1–3). Identifying neurobiological individual differences linked to early substance use may inform prevention efforts. In the present study, we examine whether differential neurobiological responsivity to social reinforcement in early adolescence is a predictor of later substance use. Reinforcing social feedback helps guide behavior and is a particularly salient natural (non-drug) reward during adolescence (4). Ventral striatal (VS) dopamine signaling plays a role in processing multiple types of reinforcement including social incentives (5). In contrast, the majority of neurons in the habenula (HB), a small bilateral, epithalamic nucleus, are excited by negative outcomes (i.e., punishments and reward omissions) but inhibited by rewards and reward predicters (6). Specifically, following unexpected negative outcomes, increased activity in the lateral HB is thought to inhibit dopaminergic midbrain neurons leading to decreased dopamine signaling in the striatum (7,8). Functional magnetic resonance imaging (fMRI) studies have repeatedly shown VS responsivity to social rewards among adolescents (9,10), and evidence in non-human primates demonstrates differential HB signaling to high and low value social stimuli (11). However, HB responses to social reinforcement have not been examined in human adolescents.

As the HB is involved in inhibiting dopamine release in the striatum (12,13), elevated HB and diminished VS responsivity may constitute a hypodopaminergic response profile. A substantial body of work has linked both heightened HB (e.g., 14,15) and blunted VS reinforcement responses to anxiety and depression symptomology (e.g., 16,17). Moreover, hypodopaminergic responses are thought to contribute to aspects of substance use disorders including drug-seeking, and withdrawal symptoms such as anhedonia, irritability, depression, anxiety, and craving (e.g., 18-24). Indeed, it is thought that continued substance use among

addicted individuals is driven, in part, by a desire to prevent or relieve such aversive withdrawal symptoms and potentially normalize differential brain functioning associated with withdrawal (25).

It is also possible that the initial propensity to use substances could be influenced by preexisting disruptions in the same functional brain mechanisms that mediate dopamine responses associated with problematic substance use. Indeed, the degree to which dysregulated brain function is caused by repeated substance exposures or is a preexisting risk-factor leading to problem use is still unclear (26,27). One approach to answering this question is examination of brain function, before the onset of heavy substance use, among individuals who will go on to increase their use in the future. There is emerging longitudinal evidence among adolescents (28) and young adults (29) indicating that individuals with relative hypoactivity of the VS during reward anticipation are more likely to engage in later substance use. Other work has shown that among individuals with no history of substance abuse, those who rated psychostimulant administration as more pleasant had lower striatal D2 receptors than those who described it as unpleasant (31). Together, these results support a neural risk factor hypothesis in which individuals with reward hyposensitivity may be prone to use substances as a means of compensating for decreased activation of reward circuits (32). However, it is important to note that extant findings relevant to this hypothesis are mixed (33). The heterogeneity of effects may be attributable to individual differences in substance use motivations and history of substance exposure, as well as differences in attentional demands invoked by incentive tasks (33).

Nevertheless, given the HB's critical role in the function of reward circuits, HB function may also be an important neurobiological marker associated with risk for later substance use. While HB function is hypothesized to be involved in the transition from positive reinforcement

mechanisms driving initial stages of recreational substance use to negative reinforcement mechanisms involved in addiction (22), links between HB reinforcement processing and progression of substance use have not been examined among adolescents.

Within the current longitudinal neuroimaging study, across a critical period of adolescence (6th through 11th grade), we investigated individual differences in developmental trajectories of HB and VS reactivity to social reinforcement in a Social Incentive Delay (SID) task. On average, we expected increased HB responsivity to negative outcomes (i.e., receipt of social punishment and omission of social reward) versus positive outcomes (i.e., receipt of social reward and avoidance of social punishment) during the SID (34-35). In contrast, we expected increased VS responsivity to positive outcomes versus negative outcomes (36). Our study design also allowed us to examine whether individual trajectories of HB and VS responses to social reinforcement across early adolescence, from 6th to 9th grade, diverged in adolescents who started using substances regularly versus those who remained abstinent throughout adolescence (6th to 11th grade). As neuroimaging research on the HB's role in substance use is still limited, and preclinical studies on substance use have observed a variety of responses among subpopulations of HB neurons (e.g., firing rates, excitatory and inhibitory transmission; 37) that blood oxygen level-dependent (BOLD) signal is unable to capture, these analyses were exploratory. Nonetheless, similar to prior theory (38), we considered relatively elevated HB and blunted VS activity to social reinforcement (i.e., receipt of social reward and omission of social punishment) to be indicative of a hypo-reward response, whereas blunted HB and elevated VS activity was considered indicative of a hyper-reward response.

METHODS

Participants. Two cohorts of adolescent participants were recruited across 2 years, from 3 public middle schools, as part of a larger study of 6th and 7th grade students. A total of 173 participants completed between 1 to 3 sessions annually across 3 waves of a longitudinal study. At each session, participants completed an fMRI scan and self-report assessments (403 total fMRI data points). 125 of these participants returned to complete a follow-up self-report session at a 4th wave, ~1 year after wave 3 and 103 returned to complete a self-report session at a 5th wave ~2 years after wave 3. The multiple cohort structure of the study resulted in planned missing data across timepoints. To assess cross-timepoint effects we utilized multi-level statistical models that allow for data missing at random. Participant recruitment, retention, and exclusions across data collection waves are reported in the supplement and data from this sample are also reported elsewhere (39-43). The final sample included 389 total fMRI data points collected from 170 individuals (53.5% female), and 228 additional self-report data points collected across waves 4 and 5.

Procedures. All participants provided informed consent/assent and were compensated for each completed session. The University's Institutional Review Board approved all aspects of the study. Adolescent participants attended annual data collection sessions, at which they completed several self-report measures and a fMRI scan lasting approximately 1.5 hours, during which they completed two runs of the SID task designed to measure neural sensitivity to anticipation and receipt of social rewards and punishments (**Figure 1**).

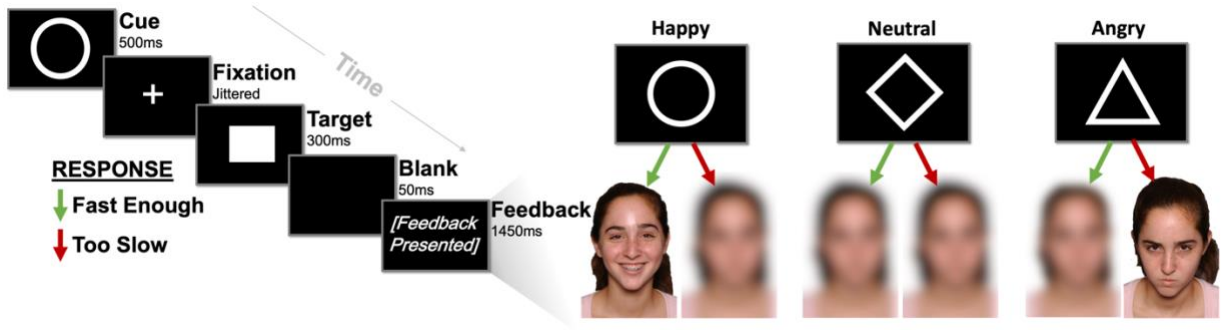


Figure 1. **Social incentive delay (SID) task**. During fMRI scanning, participants completed two 6.5 min runs of a Social Incentive Delay (SID) task designed to measure neural sensitivity to anticipation and receipt of social rewards (smiling face) and punishments (scowling face). Participants completed 116 trials (48 reward trials, 48 punishment trials, and 20 neutral trials). In the task, participants see a cue (circle, square, or diamond, 500 ms) indicating what type of trial will follow. Then, following a fixation cross (duration jittered ~509-4249 ms), they see a target (white square, 160-500 ms). Participants are trained to press their right index finger as fast as they can after seeing the target, but not before. Following a delay (50 ms), participants receive social feedback (1450 ms) based on both the trial type and whether they pressed fast enough. The social feedback is photographs of adolescent faces taken from the NIH faces dataset (45). In the task, there were 24 faces shown (12 female, 12 male). Participants are explicitly told that the circle is a happy cue (Reward Cue), the square is an angry cue (Punishment Cue), and the diamond is a neutral cue (Neutral Cue), meaning if they press fast enough after seeing the happy cue they will see a smiling face (Reward hit); if they press too slow after the happy cue, they will see blurred, noninformative face (Reward miss). If they press fast enough after seeing the angry cue, they will see a blurred, noninformative face (Punishment hit); if they press too slow after the angry cue, they will see a scowling face (Punishment miss). Following the neutral cue, they will see a blurred, noninformative face whether they press fast enough (Neutral hit) or too slow (Neutral miss). To ensure sufficient exposure to all feedback types, task difficulty was individually and dynamically adapted based on prior performance by increasing or decreasing the target duration by 20 ms intervals unless reaching a minimum of 160 ms or maximum of 500 ms duration. All participants had at least 10 instances (46) of each trial type with a mean minimum trial type occurrence of 24±3.9. To examine brain responsivity to receipt of social reinforcement, we focused on Reward hit vs. Reward miss, and Punishment hit vs. Punishment miss task contrasts.

Adolescent-reported substance use. To assess substance use we used items adapted from the standard Youth Risk Behavior Surveillance System (YRBS) which monitors categories of health-related behaviors (44). Specifically, at each wave of data collection, adolescent participants reported how many days in the last year they vaped electronic-cigarettes, smoked cigarettes, had at least one drink of alcohol, smoked marijuana, used prescription substances they did not have a

prescription for, and used other substances including crystal meth, cocaine, heroin, ecstasy, LSD, or PCP. At each wave, adolescents' maximum use of any substance that year was considered. Additionally, to index each adolescent's severity of substance use across adolescence, we also considered participants' maximum yearly substance use reported across all timepoints.

MRI data analysis. MRI data, including two functional SID task runs (voxel size = 2.5 x 2.5 x 3mm) and a T1-weighted structural image (voxel size = $0.8mm^3$), were collected on a Siemens Prisma MRI, 3-Tesla scanner and preprocessed with *fMRIPrep* v1.5.3 (47). See supplemental materials for neuroimaging data acquisition and preprocessing details. One participant was excluded due to motion. To characterize brain activity linked with delivery of social reinforcement, functional data were entered into a whole-brain, participant-level general linear model (GLM; 3dDeconvolve & 3dREMLfit) including three cue-related task regressors (Reward cue, Punishment cue, and Neutral cue) and six feedback-related task regressors (Reward hit, Reward miss, Punishment hit, Punishment miss, Neutral hit, and Neutral miss) as impulse functions time-locked to stimulus onset and convolved with a hemodynamic response (gamma) function. Regressors of no interest included six motion-correction parameters, their first derivatives, the average signal from an anatomically-derived cerebral spinal fluid (CSF) mask and its first derivative, as well as fourth-order polynomial regressors to capture baseline trends in the BOLD signal.

We utilized a region of interest (ROI) approach to assess HB and VS responsivity to task events given *a priori* hypotheses regarding the involvement of these regions in dopaminergic regulation following rewards and negative outcomes. Average β coefficients associated with task events and contrasts of interest were extracted by averaging across all voxels within co-registered bilateral HB and VS masks. The HB mask was defined by the High-resolution, Probabilistic in

vivo Anatomical Atlas of Subcortical Nuclei (48,49). The VS mask was defined by the Harvard-Oxford Structural Atlas (50). To avoid automatic partial voluming of the HB and VS regions of interest (ROIs) with surrounding tissue and CSF, spatial smoothing was not performed. Further, given the HB and VS are both adjacent to ventricles, we ensured β coefficients were only being averaged over gray-matter (GM) voxels by creating participant/session specific HB and VS ROIs. Specifically, for each participant/session, voxels in the atlas defined HB and VS masks outside each session's anatomically-derived GM mask were subtracted (**Figure 3A** & **Figure S1**). Nonetheless, given the HB's small size, distinguishing its signals from those of surrounding anatomy is difficult. Although, regionally, only the HB responds to reinforcing outcomes. Exploratory, whole-brain SID task contrast maps were also calculated (see supplemental **Figure S4**).

Statistical analysis. To assess behavioral performance in the SID task, we compared average hit rates and response times on reward versus punishment trials. To assess hypothesized differential brain responsivity to social feedback task events we conducted 2(TRIAL TYPE: reward vs. punishment) × 2(ACCURACY: hit vs. miss) repeated-measures ANOVAs on HB and VS brain activity averaged across each participant's available timepoints. As prior research suggests the HB may play a role in regulating VS responsivity to rewards and negative outcomes (51,52), we additionally conducted an exploratory psychophysiological interaction (PPI) analysis to examine task-dependent VS and HB correlations (see supplement; **Figure S5**).

To examine developmental changes in responsivity to social reinforcement, we assessed the effect of grade on HB and VS task contrast β coefficients within restricted maximum likelihood estimation (REML) linear mixed-effect models (*lmer*, R-package). The random intercept and slope of grade as well as their correlation were modeled across participants. To reduce data

dimensionality and number of statistical tests, we focused on the Reward hit vs. Reward miss and Punishment hit vs. Punishment miss task contrasts to assay social reward receipt and social punishment avoidance, respectively. Next, to assess if changes in brain responsivity differed based on participants' maximum yearly substance use, across 6th to 11th grade, we examined USE×GRADE interactions on HB and VS responsivity to the same two task contrasts, again within linear mixed-effect models allowing for random intercepts and slopes of grade as well as their correlation. Follow-up assessments of simple slopes within binned yearly substance use groups (no use; some use [1-11 days]; regular use [at least once a month]) were conducted for significant interactions.

RESULTS

Descriptives. Adolescents were from diverse racial/ethnic backgrounds and varied in socioeconomic status (SES) indicators including maternal education level and annual household income (**Table 1**). None of the adolescent participants in our sample reported regular substance use in 6th grade (*n*=85). The percent reporting some and regular substance use increased longitudinally with 2.1% reporting regular use in 7th grade (*n*=140), 8.9% reporting regular use in 8th grade (*n*=124), 13.1% reporting regular use in 9th grade (*n*=122), 20.0% reporting regular use in 10th grade (*n*=100), and 28.6% reporting regular use in 11th grade (*n*=42; **Figure 4A** & **Figure S2)**. The types of substances used by grade largely corresponded with national estimates (53,54) and are displayed in **Figure S3**. In general, alcohol and electronic-cigarettes were the most commonly reported substances across all grades, although marijuana and electronic-cigarettes were the most regularly used substances in 9th and 11th grade. Adolescents' maximum yearly substance use reported over all available timepoints was also considered. Across all timepoints,

50.3% of adolescents reported never using any substances (no use), 27.2% reported using substances 1-11 days a year (some use), and 22.5% reported using substances at least once a month for a year (regular use; **Figure 4B**).

Table 1. **Demographic and socioeconomic status information by substance use group**.

	N=170	Regular Use (n=38)	Some Use (n=46)	No Use (n=86)	Group Differences
Sex					*p*=0.7
Female	53.5%	50%	58.7%	52.3%	
Male	46.5%	50%	41.3%	47.7%	
Race/Ethnicity					*p*=0.6
Hispanic/Latinx	35.9%	47.4%	32.6%	32.6%	
White	28.8%	28.9%	32.6%	26.7%	
Black	21.8%	10.5%	26.1%	24.4%	
Multi-Racial	10.0%	10.5%	6.5%	11.6%	
Native American	2.3%	0	2.2%	3.5%	
Asian	1.2%	2.7%	0	1.2%	
Maternal Education					*p*=0.5
< Highschool diploma	25.0%	21.6%	26.1%	25.9%	
Highschool diploma	15.5%	16.2%	15.2%	15.3%	
Some college	45.3%	43.3%	41.3%	48.2%	
Bachelor's degree	10.0%	13.5%	10.9%	8.2%	
Graduate degree	4.2%	5.4%	6.5%	2.4%	
Household Annual Income					*p*=0.03*
$0-29,999	31.1%	28.9%	28.3%	33.7%	
$30,000-59,999	32.9%	28.9%	19.6%	42.2%	
$60,000-99,999	26.4%	31.6%	39.1%	16.9%	
>$100,000	9.6%	10.6%	13.0%	7.2%	

Note. Maternal education (% of total) and household annual income (% of total) were measured at each participant's first timepoint (6th or 7th grade). Substance use group differences in maternal education and household annual income were assessed with a Kruskal-Wallis test for ordinal variables which is similar to a one-way ANOVA but ranks of the data are used rather than actual data points. Substance use group × sex and substance use group × race cross tabulation Chi-squared tests were run to assess demographic group differences. Sex, race/ethnicity, and maternal education did not significantly differ across adolescent substance use groups (*p*'s>0.1). Although adolescents reporting regular, and some substance use had higher annual household incomes than adolescents reporting no substance use (*p*=0.03). As expected, at each grade, substance use groups' past year use significantly differed (*p*'s<0.001; **Figure S2**).

Habenula & ventral striatum responsivity to social reinforcement. Task behavior revealed a significantly higher hit rate (**Figure 2A**) and faster response times (**Figure 2B**) for reward trials compared to punishment trials, possibly indicating participants' greater motivation to receive social rewards than avoid social punishments. 2(TRIAL TYPE: reward vs. punishment) × 2(ACCURACY: hit vs. miss) repeated-measures ANOVAs on brain activity, averaged across each participant's available timepoints (**Figure 3B**), revealed significant TRIAL TYPE × ACCURACY interactions for both the HB ($F[1, 168]=33.4$, $p<0.001$, $\eta_p^2 = 0.17$) and the VS ($F[1, 168]=14.5$, $p<0.001$, $\eta_p^2 = 0.08$). As hypothesized, follow-up *t*-tests confirmed that, on punishment trials, the HB displayed significantly higher responsivity to miss outcomes (resulting in receipt of social punishment) compared to hit outcomes (resulting in a neutral outcome; $t[168]=-6.0$, $p<0.001$, $d=-0.5$). However, on reward trials, contrary to hypotheses, the HB displayed increased responsivity to hit outcomes (resulting in receipt of social reward) versus miss outcomes (resulting in a neutral outcome; $t[168]=2.5$, $p=0.015$, $d=0.2$). The VS displayed expected increased responsivity to hit vs. miss outcomes on both reward trials ($t[168]=17.8$, $p<0.001$, $d=1.4$) and punishment trials ($t[168]=-6.1$, $p<0.001$, $d=-0.5$), indicating greater activity to receiving social rewards and avoiding social punishments. These results were further supported by exploratory whole-brain *t*-tests for each contrast of interest which demonstrated decreased activity in small clusters overlapping with habenular nuclei to social punishment hit vs. miss outcomes but increased HB activity to social reward hit vs. miss outcomes (**Figure S4**).

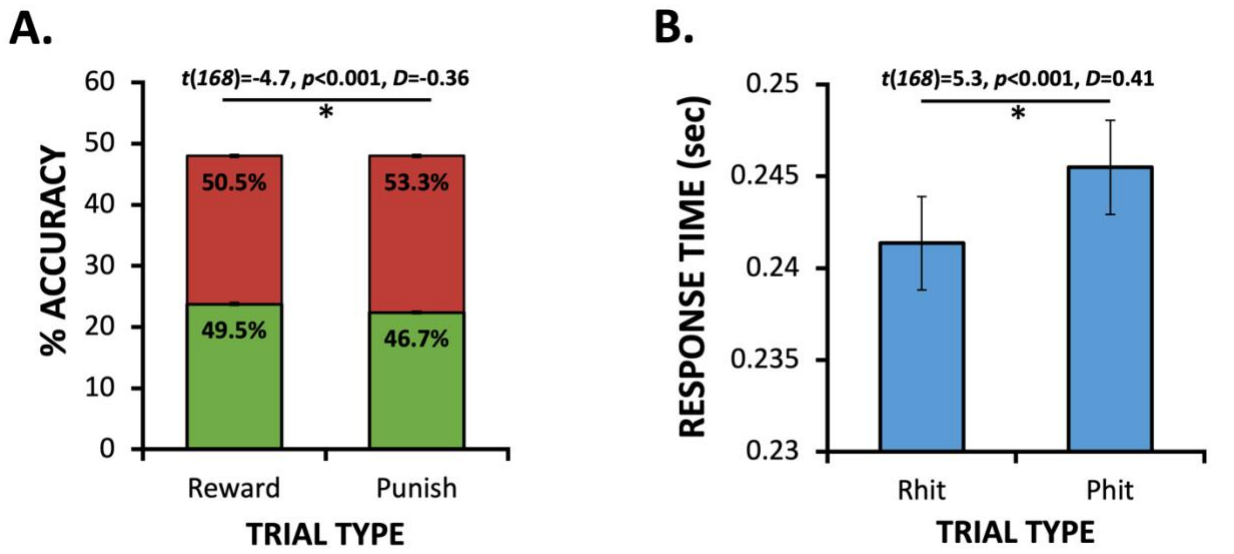


Figure 2. **Social incentive delay (SID) task behavior**. Task behavior was averaged across all timepoints for each participant. **(A)** Hit rate (percent of correct responses) for reward trials (49.5%) was significantly higher than the hit rate for punishment trials (46.7%; $t[168]$=-4.7, p<0.001, d=-0.36). **(B)** Additionally, average response time for hits on reward trials (0.24sec ± 0.003sec) was significantly faster than response time for hits on punishment trials (0.25sec ± 0.003sec; $t[168]$=5.3, p<0.001, d=0.41). Overall, task behavior suggests that, even with an individualized difficulty manipulation in place, there was still some variability in task performance such that participants may have been more motivated to correctly hit the target on reward trials and receive social rewards than correctly hit the target on punishment trials and avoid social punishments.

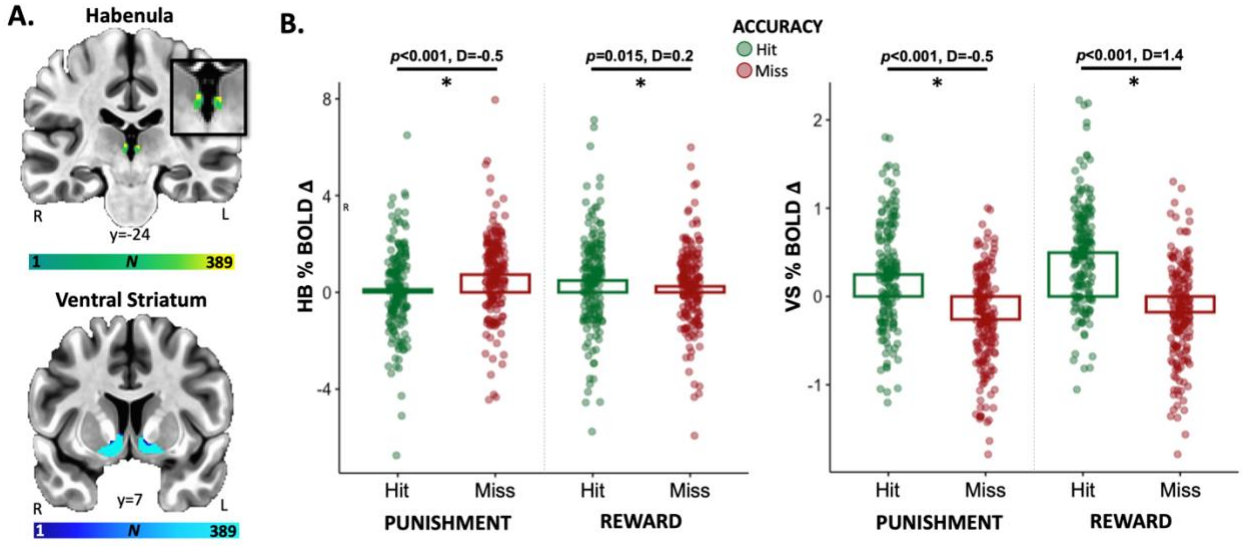


Figure 3. **Differential habenula & ventral striatum responsivity to social reinforcement. (A)** To ensure that region of interest (ROI) β coefficients were extracted only from gray matter voxels (as opposed to those in cerebral spinal fluid [CSF] or white matter), we defined participant and

session specific habenula (HB) and ventral striatum (VS) ROIs by subtracting voxels from atlas defined masks that were outside each participant's and session's anatomically-derived gray matter mask. GM masks were generated via brain tissue segmentation using fast (FSL 5.0.9) within the standard *fMRIPrep* pipeline. Across all participants and sessions, an average of 6.6 ± 2.8 voxels were removed from the bilateral HB mask and an average of 21.9 ± 15.4 voxels were removed from the bilateral VS mask resulting in the HB ROIs being 7.4 ± 2.8 voxels on average and the VS ROIs being 331.1 ± 15.4 voxels on average. ROI size was not correlated with its β coefficient (p>0.2). These participant/session specific ROIs were summed to produce an overlap image with voxel values ranging from 0 to 389 (total number of fMRI timepoints) that represent the distribution of HB and VS ROI sizes across all participants and sessions. **(B)** Significant TRIAL TYPE × ACCURACY interactions for both the HB and VS. On punishment trials, the HB displayed significantly higher responsivity to miss outcomes compared to hit outcomes. However, on reward trials, contrary to hypotheses, the HB displayed increased responsivity to hit outcomes versus miss outcomes. The VS displayed expected increased responsivity to hit vs. miss outcomes on both reward trials and punishment trials.

Developmental trajectory of habenula and ventral striatum responsivity based on substance use. We did not observe significant longitudinal changes in HB reward hit vs. miss or punishment hit vs. miss task contrast β coefficients across grades (p>0.8). Nor did VS responsivity to punishment hit vs. miss outcomes significantly change over grade (p>0.8). However, VS responsivity to reward hit vs. miss outcomes displayed a significant within-person effect of grade such that activity decreased longitudinally from 6th to 9th grade (β = -0.13 ± 0.1, p = 0.022; **Figure S6**). Quadratic models did not provide a significantly better model fit for the relationships than the linear models (p's>0.9).

Moreover, changes in brain responsivity across grade differed based on adolescents' maximum yearly substance use. Significant USE×GRADE interactions on HB responsivity to social reward hit vs. miss outcomes (p=0.035; **Figure 4C**) indicated that adolescents reporting regular substance use displayed relatively higher HB responsivity to social reward outcomes in 6th grade that declined longitudinally. In contrast, adolescents reporting no substance use displayed relatively lower HB responsivity in 6th grade that increased longitudinally through 9th grade. Further, significant USE×GRADE interactions on VS responsivity to social punishment hit vs.

miss outcomes (p=0.039; **Figure 4D**) indicated that adolescents reporting regular substance use displayed longitudinal increases in VS responsivity whereas, adolescents reporting none and some substance use had relatively stable VS responsivity over this time frame. These results remained significant when controlling for SES. No significant USE×GRADE interactions were observed for HB responsivity to social punishment hit vs. miss outcomes (p>0.3) or for VS responsivity to social reward hit vs. miss outcomes (p>0.3).

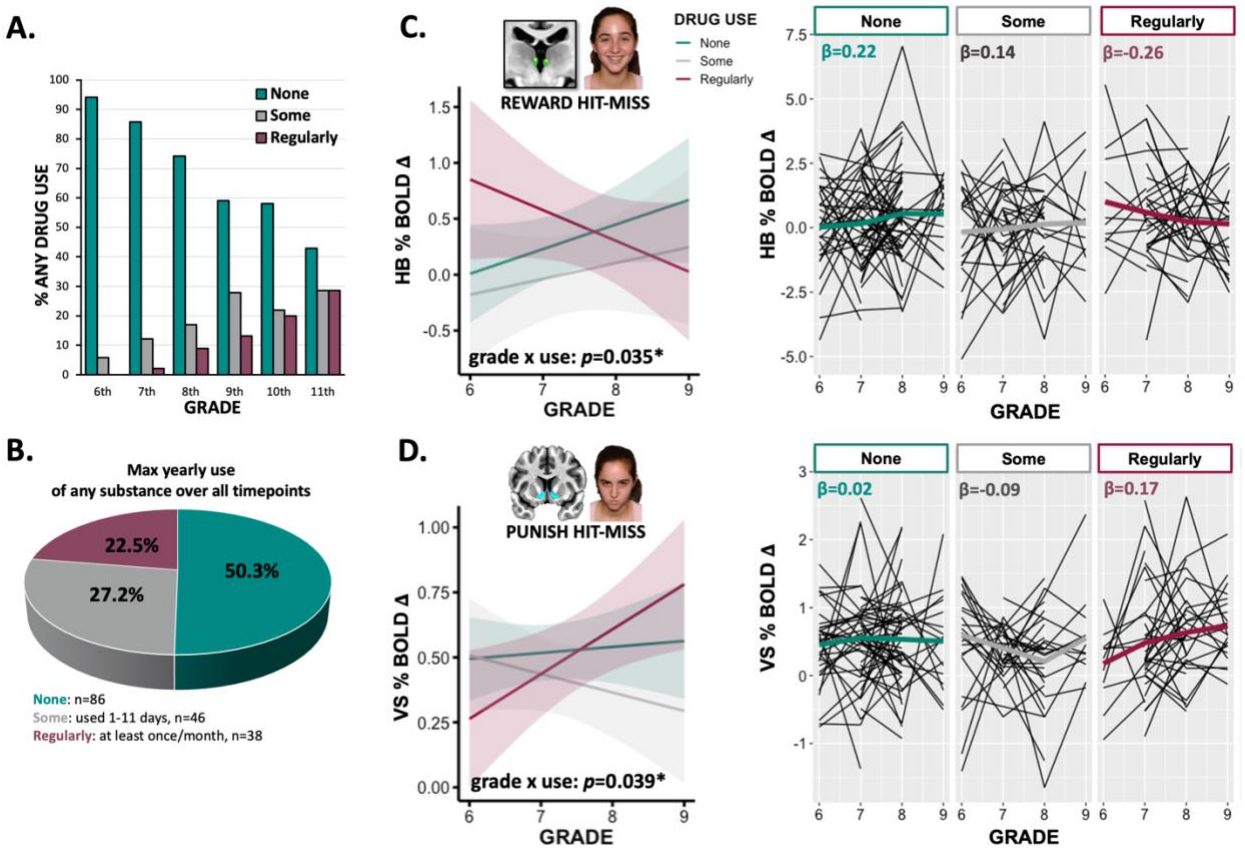


Figure 4. Developmental trajectory of habenula and ventral striatum responsivity based on substance use. (A) Percent of participants in each grade reporting none, some, or regular use of any substance in the past year (**None**: teal; no reported use in the last year, **Some**: gray; use of any substance on 1-11 days in the last year; **Regularly**: pink; use of any substance at least once a month or more in the last year). The percent of participants reporting some and regular substance use increased over grade with no participants reporting regular use in 6th grade (n=85), 2.1% reporting regular use in 7th grade (n=140), 8.9% reporting regular use in 8th grade (n=124), 13.1% reporting regular use in 9th grade (n=122), 20.0% reporting regular use in 10th grade (n=100), and 28.6%

reporting regular use in 11th grade (n=42). **(B)** Percent of sample in each substance use group. Each subject's maximum yearly use of any substances over all grades was used to determine their substance use group. **(C)** Significant USE×GRADE interaction on habenula (HB) responsivity to social reward hit vs. miss outcomes (t[*318.1*]=-2.2, β=-0.08, p=0.035). Follow-up tests of simple slopes within substance use groups indicated that adolescents reporting regular substance use displayed relatively higher HB responsivity to reward hit vs. miss outcomes in 6th grade (B_0=0.8±0.4) that declined as grade increased (B_1=-0.3, t[*35.1*]=-1.2, p=0.2). In contrast, HB responsivity to reward hit vs. miss outcomes among adolescents reporting some substance use (B_1=0.1, t[*33.4*]=0.7, p=0.5), and no substance use (B_1=0.2, t[*141.9*]=1.6, p=0.1) was relatively lower in 6th grade and increased longitudinally. **(D)** Significant USE×GRADE interaction on ventral striatum (VS) responsivity to social punishment hit vs. miss outcomes (t[*127.2*]=2.1, β=0.03, p=0.039). Adolescents reporting regular substance use displayed relatively lower VS responsivity to social punishment hit vs. miss outcomes in 6th grade (B_0=0.3±0.1) that significantly increased longitudinally (B_1=0.2, t[*92.1*]=2.4, p=0.02), whereas adolescents reporting some substance use displayed VS responsivity that was relatively higher in 6th grade (B_0=0.5±0.1) and decreased longitudinally (B_1=-0.09, t[*31.4*]=-1.1, p=0.3). Adolescents reporting no substance use also displayed VS responsivity that was relatively higher in 6th grade (B_0=0.5±0.08) but that did not change longitudinally (B_1=-0.02, t[*135.3*]=-0.4, p=0.7). No significant USE×GRADE interactions were observed for HB responsivity to social punishment hit vs. miss outcomes (p>0.3) or VS responsivity to social reward hit vs. miss outcomes (p>0.3).

DISCUSSION

Adolescence is a critical period when initial substance use often transitions to more regular use (1–3). Within a longitudinal design, across a critical period for substance use onset (6th through 11th grade), we investigated individual differences in developmental trajectories of HB and VS social reinforcement processing associated with substance use. Adolescents reporting regular use (at least once a month over a year) at any point during adolescence displayed relatively higher HB responsivity to the receipt of social rewards in 6th grade (before any regular substance use was reported) that significantly declined longitudinally. In contrast, adolescents reporting little (1-11 day in a year), or no use, displayed relatively lower HB responsivity in 6th grade that did not decrease as grade increased. Notably, we observed the opposite pattern for VS responsivity to the avoidance of social punishment such that adolescents reporting regular substance use displayed longitudinally increasing VS responsivity. These results identify individual differences in social

reinforcement processing trajectories across adolescence that are associated with substance use behaviors.

Differential habenula & ventral striatum responsivity to social reinforcement. We specifically focused on VS and HB responsivity to receipt of positive social outcomes (i.e., receipt of social rewards and avoidance of social punishment) verses receipt of negative social outcomes (i.e., omission of social rewards and receipt of social punishment), given that VS dopamine signaling plays a role in processing rewards, whereas HB neurons are inhibited by rewards but excited by negative outcomes (i.e., punishments and reward omissions) (6). As expected, the VS displayed increased responsivity to hit vs. miss outcomes on both reward and punishment trials. Further, we confirmed hypothesized increased HB responsivity to miss outcomes (receipt of social punishment) compared to hit outcomes (avoidance of social punishment) on punishment trials. Our exploratory PPI analyses (see supplemental materials) furthermore found that HB and VS activity was more likely to be negatively correlated during any kind of miss vs. hit feedback, regardless of the valence of those outcomes. Taken together, these findings may reflect the HB's role in downregulating striatal responses to negative and unexpected outcomes (51-52). However, on reward trials, contrary to hypotheses, the HB displayed increased responsivity to hit outcomes (social reward) versus miss (neutral) outcomes. These observations were not expected but may reflect limitations of the BOLD signal in distinguishing between initial inhibitory and rebound excitatory HB reward responses that have been observed in some preclinical studies (6,55-56). More translational work is needed to understand how habenula reward responses are exhibited in BOLD activty. Another possible explanation of this unexpected finding is that the HB may be responsive to all social stimuli (vs. neutral, non-informative feedback) regardless of valence. Given that emerging findings among animal models have demonstrated the HB's role when engaging

with social conspecifics across aversive and rewarding outcomes (6,57,58), our results may show initial support for a similar role among humans. Nonetheless, our results suggest that social punishments, in the SID task, elicit adolescent VS and HB activation similar to other feedback processing tasks (18,34,36).

Developmental trajectory of habenula and ventral striatum responsivity based on substance use. Results delineated individual differences in social reinforcement processing trajectories across adolescence that are associated with substance use. These findings suggest that initially elevated and longitudinally decreasing HB, but blunted and longitudinally increasing VS, responses to social reinforcement (i.e., receipt of social reward and omission of social punishment) may be associated with substance use in adolescence. Whereas prior work suggests that elevated HB and blunted VS responses to reinforcement may constitute a hypodopaminergic response profile (59), BOLD signal is not specific to any one neurochemical system and thus future pharmaco-imaging studies are needed to interrogate the dopaminergic nature of these responses. Nonetheless, our results build upon recent longitudinal evidence implicating decreased reinforcement-related striatal function in risk for later substance use (28,29) by also linking initially elevated and longitudinally decreasing activity in a brain region, known to be involved in inhibiting dopamine release in the striatum (i.e., HB), with substance use.

One hypothesis suggests that the relatively elevated HB and blunted VS responses we observed in 6th grade are reflective of relative dysregulation of reward processing and that substance use may be a means of compensating for decreased activation of reward circuits among individuals displaying reward hyposensitivity (28-32). Our results further support this theory, demonstrating progressive decreases in initial reward hyposensitivity over years of increasing substance use among the adolescents who become regular substance users (at least once a month

use over a year) from grade 6th to 11th grade. In contrast, adolescents who maintained only moderate substance use (less than once a month) across 6th to 11th grades did not show the same longitudinal decreases in reward hyposensitivity. These findings suggest that those who show initially elevated HB and blunted VS responses may be prone to trying substances, while those who go on to use substances more regularly, transition to a more hyper-VS and hypo-HB response. As a substantial body of work links both heightened HB and blunted VS reward responses to anhedonia and substance craving (14-16,60,61), decreases in these initial responses among regular users could possibly suggest a self-medication explanation of use escalation over grades. However, it is worth noting that some prior work has, in contrast, hypothesized hyper-responsivity in mesolimbic reward circuitry among at-risk, drug-naive youth, that transitions to hypo-responsivity once regular substance use has commenced (33). As such, it's possible that different sub-populations of adolescents have different motivations for engaging in substance use, and possibly different respective neural responses to reinforcement (33). More work is needed to assess our explanation.

While our findings implicate neural sensitivity to social feedback (smiling and scowling adolescent faces) in risk for adolescent substance use, we were not able to dissociate these effects from that of brain responsivity to nonsocial reinforcement outcomes. Nonetheless, social reinforcement guides behavior, is particularly salient during adolescence (5,6), and existing research has repeatedly highlighted the role of peer influence in adolescent substance use (e.g., 62-64). Future research should consider how neurobiological sensitivities to social feedback in general, might contribute to adolescents' susceptibility to peer influence and, in turn, substance use (65). Indeed, existing work has shown that individual differences in VS activity interacts with peer norms to predict later risk behavior (including substance use) (40). Again, in light of these

prior findings and our current results, there are likely differential pathways to substance use (i.e., substance use risk due to high valuation of peer feedback vs. self-medication of anhedonia). As a combination of interacting genetic and environmental factors likely contribute to the propensity to display these neurobiological response patterns in early adolescence (66), causes of these individual differences should also be considered in future work. Specifically, as prior studies suggest that early life adversity is associated with both altered neural reinforcement processing (67,68) and substance use (69), potentially confounding effects of these experiences should be examined.

Limitations. Our findings provide new evidence of social reinforcement brain activity linked to substance use in a relatively large sample of adolescents from racially and socioeconomically diverse backgrounds. However, certain limitations should be considered. First, our sample focused on adolescents ages 10–17 years old. We focused on this age range since adolescence is marked by a social reorientation towards peers, increases in reward processing relative to children and adults, and the onset of substance use (62-65). However, future research should consider whether brain activity related to social reinforcement, at either earlier or later ages, is similarly linked to substance use, or whether these findings are unique to the timeframe examined. Second, as this study did not include a non-social incentive delay task, we were unable to determine specificity of social versus non-social reinforcement brain responsivity effects on substance use outcomes. Third, we utilized adolescents' grade to examine developmental effects since youth's responsivity to social reinforcement may be more contingent on social experiences linked to their grade level in school. However, future investigations should also consider other metrics of development, like age or pubertal development. Finally, at the resolution of fMRI studies, medial and lateral subregions of the HB cannot be dissociated and given the HB's small

size (~36mm^3), distinguishing HB signals from those of the surrounding thalamus is difficult (70, 71). We acknowledge that the fMRI signal from such a small anatomical region may be contaminated by surrounding non-HB signals (e.g., other thalamic regions, physiologic noise). However, regionally only the HB responds to reinforcing outcomes (71,34).

Conclusion. Taken together, these results identified individual differences in social reinforcement processing trajectories across adolescence associated with substance use. Specifically, initially elevated HB and blunted VS responses to social reinforcement (receipt of social reward and omission of social punishment) may be risk factors for substance use, while progressive decreases in these responses may be associated with use escalation. Identifying adolescents most at risk for substance use, before the onset of regular use, as well as neurobiological mechanisms that may contribute to use progression, could inform prevention efforts, and help identify critical targets for interventions.

ACKNOWLEDGMENTS: Primary support for this project was provided by grants from the National Institute on Drug Abuse (NIDA) [R01DA051127] to EHT & KAL, and [R01DA039923] to EHT.

CODE & DATA AVAVAILABILITY: The authors have released all code associated with this manuscript. Code and tabular data are available on GitHub (https://github.com/Flanneryg3/DevHB_ProjectCode).

DISCLOSURES: The authors have no conflicts to declare.

REFERENCES

1. Wagner FA, Anthony JC. From First Drug Use to Drug Dependence: Developmental Periods of Risk for Dependence upon Marijuana, Cocaine, and Alcohol. Neuropsychopharmacology. 2002 Apr 1;26(4):479–88.

2. DeWit DJ, Adlaf EM, Offord DR, Ogborne AC. Age at First Alcohol Use: A Risk Factor for the Development of Alcohol Disorders. Am J Psychiatry. 2000 May;157(5):745–50.

3. Kandel DB, Logan JA. Patterns of Drug Use from Adolescence to Young Adulthood: 1. Periods of Risk for Initiation, Continued Use, and Discontinuation. 1984;74(7):7.

4. Do KT, Prinstein MJ, Telzer EH. Neurobiological susceptibility to peer influence in adolescence. In: Cohen Kadosh K, editor. The Oxford Handbook of Developmental Cognitive Neuroscience. Oxford University Press, 2020.

5. Izuma K, Saito DN, Sadato N. Processing of the Incentive for Social Approval in the Ventral Striatum during Charitable Donation. J Cogn Neurosci. 2010 Apr 1;22(4):621–31.

6. Mondoloni S, Mameli M, Congiu M. Reward and aversion encoding in the lateral habenula for innate and learned behaviours. Transl Psychiatry. 2022 Jan 10;12(1):1–8.

7. Velasquez K, Molfese D, Salas R. The role of the habenula in drug addiction. Front Hum Neurosci. 2014;8.

8. Hikosaka O. The habenula: from stress evasion to value-based decision-making. Nat Rev Neurosci. 2010 Jul;11(7):503–13.

9. Somerville LH, Hare T, Casey BJ. Frontostriatal Maturation Predicts Cognitive Control Failure to Appetitive Cues in Adolescents. J Cogn Neurosci. 2011 Sep 1;23(9):2123–34.

10. Pfeifer JH, Masten CL, Moore WE, Oswald TM, Mazziotta JC, Iacoboni M, et al. Entering Adolescence: Resistance to Peer Influence, Risky Behavior, and Neural Changes in Emotion Reactivity. Neuron. 2011 Mar 10;69(5):1029–36.

11. Lee H, Hikosaka O. Lateral Habenula Responses During Eye Contact in a Reward Conditioning Task. Front Behav Neurosci. 2022 Mar 14;16:815461.

12. Hikosaka O, Sesack SR, Lecourtier L, Shepard PD. Habenula: Crossroad between the Basal Ganglia and the Limbic System. J Neurosci. 2008 Nov 12;28(46):11825–9.

13. Lecourtier L, DeFrancesco A, Moghaddam B. Differential tonic influence of lateral habenula on prefrontal cortex and nucleus accumbens dopamine release. Eur J Neurosci. 2008;27(7):1755–62.

14. Lawson RP, Nord CL, Seymour B, Thomas DL, Dayan P, Pilling S, et al. Disrupted habenula function in major depression. Mol Psychiatry. 2017 Feb;22(2):202–8.

15. Yoshino A, Aizawa H, Takamura M, Ichikawa N, Shibasaki C, Yamawaki Y, et al. [The Role of the Habenula in Depression: A Review of the Human fMRI Studies]. Brain Nerve Shinkei Kenkyu No Shinpo. 2018 Sep 1;70(9):1017–23.

16. Hanson JL, Hariri AR, Williamson DE. Blunted Ventral Striatum Development in Adolescence Reflects Emotional Neglect and Predicts Depressive Symptoms. Biol Psychiatry. 2015 Nov 1;78(9):598–605.

17. Luking KR, Pagliaccio D, Luby JL, Barch DM. Depression Risk Predicts Blunted Neural Responses to Gains and Enhanced Responses to Losses in Healthy Children. J Am Acad Child Adolesc Psychiatry. 2016 Apr;55(4):328–37.

18. Flannery JS, Riedel MC, Poudel R, Laird AR, Ross TJ, Salmeron BJ, et al. Habenular and striatal activity during performance feedback are differentially linked with state-like and trait-like aspects of tobacco use disorder. Sci Adv. 2019;5(10): eaax2084.

19. Lesage E, Aronson SE, Sutherland MT, Ross TJ, Salmeron BJ, Stein EA. Neural Signatures of Cognitive Flexibility and Reward Sensitivity Following Nicotinic Receptor Stimulation in Dependent Smokers: A Randomized Trial. JAMA Psychiatry. 2017 Jun 1;74(6):632–40.

20. Martz ME, Trucco EM, Cope LM, Hardee JE, Jester JM, Zucker RA, et al. Association of Marijuana Use With Blunted Nucleus Accumbens Response to Reward Anticipation. JAMA Psychiatry. 2016 Aug 1;73(8):838–44.

21. Mathis V, Kenny PJ. From controlled to compulsive drug-taking: The role of the habenula in addiction. Neurosci Biobehav Rev. 2019 Nov 1;106:102–11.

22. Batalla A, Homberg JR, Lipina TV, Sescousse G, Luijten M, Ivanova SA, et al. The role of the habenula in the transition from reward to misery in substance use and mood disorders. Neurosci Biobehav Rev. 2017 Sep 1;80:276–85.

23. McLaughlin I, Dani JA, De Biasi M. The medial habenula and interpeduncular nucleus circuitry is critical in addiction, anxiety, and mood regulation. J Neurochem. 2017;142(S2):130–43.

24. Pang X, Liu L, Ngolab J, Zhao-Shea R, McIntosh JM, Gardner PD, et al. Habenula cholinergic neurons regulate anxiety during nicotine withdrawal via nicotinic acetylcholine receptors. Neuropharmacology. 2016 Aug 1;107:294–304.

25. Koob GF, Le Moal M. Plasticity of reward neurocircuitry and the "dark side" of drug addiction. Nat Neurosci. 2005 Nov;8(11):1442–4.

26. Koob GF, Volkow ND. Neurocircuitry of Addiction. Neuropsychopharmacology. 2010 Jan;35(1):217–38.

27. Kalivas PW, Brady K. Getting to the core of addiction: Hatching the addiction egg. Nat Med. 2012 Apr;18(4):502–3.

28. Büchel C, Peters J, Banaschewski T, Bokde ALW, Bromberg U, Conrod PJ, et al. Blunted ventral striatal responses to anticipated rewards foreshadow problematic drug use in novelty-seeking adolescents. Nat Commun. 2017 Feb 21;8(1):14140.

29. Bart CP, Nusslock R, Ng TH, Titone MK, Carroll AL, Damme KSF, et al. Decreased reward-related brain function prospectively predicts increased substance use. J Abnorm Psychol. 2021;130(8):886–98.

30. Glick SD, Raucci J, Wang S, Keller Jr RW, Carlson JN. Neurochemical predisposition to self-administer cocaine in rats: individual differences in dopamine and its metabolites. Brain Research. 1994 Aug ; 653(1-2):148-54.

31. Volkow ND, Wang GJ, Fowler JS, Logan J, Gatley SJ, Gifford A, et al. Prediction of Reinforcing Responses to Psychostimulants in Humans by Brain Dopamine D2 Receptor Levels. Am J Psychiatry. 1999 Sep;156(9):1440–3.

32. Volkow ND, Fowler JS, Wang GJ. Role of dopamine in drug reinforcement and addiction in humans: results from imaging studies. Behav Pharmacol. 2002 Sep;13(5):355–66.

33. Bjork JM. The ups and downs of relating nondrug reward activation to substance use risk in adolescents. Curr Addict Rep. 2020 Sep;7(3):421–9.

34. Ullsperger M, Cramon DY von. Error Monitoring Using External Feedback: Specific Roles of the Habenular Complex, the Reward System, and the Cingulate Motor Area Revealed by Functional Magnetic Resonance Imaging. J Neurosci. 2003 May 15;23(10):4308–14.

35. Salas R, Baldwin P, De Biasi M, Montague R. BOLD responses to negative reward prediction errors in human habenula. Front Hum Neurosci. 2010;4.

36. Delgado MR, Nystrom LE, Fissell C, Noll DC, Fiez JA. Tracking the Hemodynamic Responses to Reward and Punishment in the Striatum. J Neurophysiol. 2000 Dec;84(6):3072–7.

37. Graziane NM, Neumann PA, Dong Y. A Focus on Reward Prediction and the Lateral Habenula: Functional Alterations and the Behavioral Outcomes Induced by Drugs of Abuse. *Frontiers in Synaptic Neuroscience*. 2018;10:12.

38. Batalla A, Homberg JR, Lipina TV, et al. The role of the habenula in the transition from reward to misery in substance use and mood disorders. *Neuroscience & Biobehavioral Reviews*. 2017;80:276-285. doi:10.1016/j.neubiorev.2017.03.019

39. Jorgensen NA, Muscatell KA, McCormick EM, Prinstein MJ, Lindquist KA, Telzer EH. Neighborhood Disadvantage, Race/Ethnicity, and Neural Sensitivity to Social Threat and Reward among Adolescents. Soc Cogn Affect Neurosci. 2022 Sep 30;nsac053.

40. Telzer EH, Jorgensen NA, Prinstein MJ, Lindquist KA. Neurobiological Sensitivity to Social Rewards and Punishments Moderates Link Between Peer Norms and Adolescent Risk Taking. Child Dev. 2021;92(2):731–45.

41. Maza MT, Fox KA, Kwon S, Flannery JE, Lindquist KA, Prinstein MJ, Telzer EH. Habitual checking behaviors on social media relate to longitudinal functional brain development. JAMA Pediatrics. in press.

42. Pollak OH, Kwon SJ, Jorgensen NA, Lindquist KA, Telzer EH, & Prinstein MJ. Neural reactivity to social punishment predicts future engagement in nonsuicidal self-injury among peer-rejected adolescents. Bio Psychiatry. in press.

43. Turpyn CC, Jorgensen, NA, Prinstein, MJ, Lindquist KA, Telzer EH. Social neural sensitivity as a susceptibility marker to family context in predicting adolescent externalizing behavior. Dev Cog Neurosci. 2021; 51, 100993-100993.

44. Kann L, McManus T, Harris WA, Shanklin SL, Flint KH, Hawkins J, et al. Youth Risk Behavior Surveillance — United States, 2015. Morb Mortal Wkly Rep Surveill Summ. 2016;65(6):1–174.

45. Egger HL, Pine DS, Nelson E, Leibenluft E, Ernst M, Towbin KE, et al. The NIMH Child Emotional Faces Picture Set (NIMH-ChEFS): a new set of children's facial emotion stimuli: Child emotional faces stimuli. Int J Methods Psychiatr Res. 2011 Sep;20(3):145–56.

46. Steele VR, Anderson NE, Claus ED, Bernat EM, Rao V, Assaf M, et al. Neuroimaging measures of error-processing: Extracting reliable signals from event-related potentials and functional magnetic resonance imaging. NeuroImage. 2016 May;132:247–60.

47. Esteban O, Markiewicz CJ, Blair RW, Moodie CA, Isik AI, Erramuzpe A, et al. fMRIPrep: a robust preprocessing pipeline for functional MRI. Nat Methods. 2019 Jan;16(1):111–6.

48. Tyszka JM, Pauli W, Nili A, Okamoto M. Reinforcement Learning Atlas. 2017 Apr 14; DOI:10.17605/OSF.IO/JKZWP, ARK: c7605/osf.io/jkzwp, Available from: https://osf.io/jkzwp/

49. Pauli WM, Nili AN, Tyszka JM. A high-resolution probabilistic in vivo atlas of human subcortical brain nuclei. Sci Data. 2018 Dec;5(1):180063.

50. Harvard-Oxford cortical and subcortical structural atlases (RRID:SCR_001476). Available from: http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases, https://neurovault.org/collections/262/

51. Baker PM, Mizumori SJY. Control of behavioral flexibility by the lateral habenula. Pharmacol Biochem Behav. 2017 Nov 1;162:62–8.

52. Kawai T, Yamada H, Sato N, Takada M, Matsumoto M. Roles of the Lateral Habenula and Anterior Cingulate Cortex in Negative Outcome Monitoring and Behavioral Adjustment in Nonhuman Primates. Neuron. 2015 Nov 18;88(4):792–804.

53. Creamer MR, Everett Jones S, Gentzke AS, Jamal A, King BA. Tobacco Product Use Among High School Students — Youth Risk Behavior Survey, United States, 2019. MMWR Suppl. 2020 Aug 21;69(1):56–63.

54. Jones CM, Clayton HB, Deputy NP, Roehler DR, Ko JY, Esser MB, et al. Prescription Opioid Misuse and Use of Alcohol and Other Substances Among High School Students — Youth Risk Behavior Survey, United States, 2019. MMWR Suppl. 2020 Aug 21;69(1):38–46.

55. Li J, Fan R, Liu X, Shen X, Liu X, Zhao H. The convergence of aversion and reward signals in individual neurons of the mice lateral habenula. Exp Neurol. 2021 May 1;339:113637.

56. Moon HS, Jiang H, Vo TT, Jung WB, Vazquez AL, Kim SG. Contribution of Excitatory and Inhibitory Neuronal Activity to BOLD fMRI. Cereb Cortex. 2021 Sep 1;31(9):4053–67.

57. Benekareddy M, Stachniak TJ, Bruns A, Knoflach F, von Kienlin M, Künnecke B, et al. Identification of a Corticohabenular Circuit Regulating Socially Directed Behavior. Biol Psychiatry. 2018 Apr 1;83(7):607–17.

58. Valentinova K, Tchenio A, Trusel M, Clerke JA, Lalive AL, Tzanoulinou S, et al. Morphine withdrawal recruits lateral habenula cytokine signaling to reduce synaptic excitation and sociability. Nat Neurosci. 2019 Jul;22(7):1053–6.

59. Lecca S, Melis M, Luchicchi A, Muntoni AL, Pistis M. Inhibitory Inputs from Rostromedial Tegmental Neurons Regulate Spontaneous Activity of Midbrain Dopamine Cells and Their Responses to Drugs of Abuse. Neuropsychopharmacology. 2012 Apr;37(5):1164–76.

60. Liu WH, Valton V, Wang LZ, Zhu YH, Roiser JP. Association between habenula dysfunction and motivational symptoms in unmedicated major depressive disorder. Soc Cogn Affect Neurosci. 2017 Sep 1;12(9):1520–33.

61. Der-Avakian A, Markou A. The neurobiology of anhedonia and other reward-related deficits. Trends Neurosci. 2012 Jan 1;35(1):68–77.

62. Simons-Morton B. Social Influences on Adolescent Substance Use. Am J Health Behav. 2007 Nov 1;31(6):672–84.

63. Liu J, Zhao S, Chen X, Falk E, Albarracín D. The influence of peer behavior as a function of social and cultural closeness: A meta-analysis of normative influence on adolescent smoking initiation and continuation. Psychol Bull. 2017;143(10):1082–115.

64. McMillan C, Felmlee D, Osgood DW. Peer influence, friend selection, and gender: How network processes shape adolescent smoking, drinking, and delinquency. Soc Netw. 2018 Oct 1;55:86–96.

65. Allen JP, Chango J, Szwedo D, Schad M, Marston E. Predictors of Susceptibility to Peer Influence Regarding Substance Use in Adolescence. Child Dev. 2012;83(1):337–50.

66. Enoch MA. Genetic and Environmental Influences on the Development of Alcoholism. Ann N Y Acad Sci. 2006;1094(1):193–201.

67. Richter A, Krämer B, Diekhof EK, Gruber O. Resilience to adversity is associated with increased activity and connectivity in the VTA and hippocampus. NeuroImage: Clinical. 2019 Jan 1;23:101920.

68. Hendrikse CJ, du Plessis S, Luckhoff HK, Vink M, van den Heuvel LL, Scheffler F, Phahladira L, Smit R, Asmal L, Seedat S, Emsley R. Childhood trauma exposure and reward processing in healthy adults: A functional neuroimaging study. Journal of neuroscience research. 2022 Jul;100(7):1452-62.

69. De la Peña-Arteaga V, Nogueira SO, Lynskey M, Hines LA. The relationship between childhood physical and sexual abuse and adolescent cannabis use: A systematic review. Frontiers in psychiatry. 2021 May 26;12:631245.

70. Lawson RP, Drevets WC, Roiser JP. Defining the habenula in human neuroimaging studies. NeuroImage. 2013 Jan 1;64:722–7.

71. Epstein EL, Hurley RA, Taber KH. The Habenula's Role in Adaptive Behaviors: Contributions From Neuroimaging. J Neuropsychiatry Clin Neurosci. 2018 Jan;30(1):A4-4.

SUPPLEMENTAL INFORMATION

Developmental changes in habenular and striatal social reinforcement responsivity across adolescence linked with substance use.

Jessica S. Flannery[1], Nathan A. Jorgensen[1], Seh-Joo Kwon[1], Mitchell J. Prinstein[1], Eva H. Telzer[1], Kristen A. Lindquist[1]

[1]Department of Psychology & Neuroscience, University of North Carolina at Chapel Hill, Chapel Hill, NC

SUPPLEMENTAL CONTENT

Retention and demographic info for data collection waves. Participants were recruited from a larger study of 873 students in 6th and 7th grade in a small, diverse, rural community in the southeast United States. Data collection took place from December 2016 to January 2022. Demographic, descriptive, and fMRI data from this sample are also reported elsewhere (1,2). At wave 1, five participants were excluded due to exclusionary criteria being met after recruitment (e.g., major claustrophobia during the fMRI session). These participants were not invited back for subsequent study participation. Out of the 143 remaining wave 1 participants, 8 were excluded due to impeded task data collection (e.g., acute participant anxiety, overwritten E-prime file). The final wave 1 sample size included 135 adolescents with 66 (48.9%) in 6th grade and 69 (51.1%) in 7th grade (Mage = 12.8±0.5; 70 [51.9%] female). At wave 2, 116 participants from cohort 1, and 30 new participants from cohort 2, participated. 17 participants were excluded due to impeded task data collection, resulting in 129 adolescents included in wave 2 with 19 (14.7%) in 6th grade, 59 (45.7%) in 7th grade, and 51 (39.5%) in 8th grade (Mage = 13.7±0.6; 67 [51.9%] female). Finally, at wave 3, 119 participants from cohort 1, and 26 participants from cohort 2, participated. 20 participants were excluded due to impeded task data collection bringing the final wave 3 sample size to 125 adolescents with 15 (11.6%) in 7th grade, 59 (45.7%) in 8th grade, and 51 (39.5%) in 9th grade (Mage = 14.7±0.6; 61 [47.3%] female). 125 additional substance use self-report data points were collected at wave 4 with 14 (40.0%) in 8th grade, 60 (48.0%) in 9th grade, and 50 (40.0%) in 10th grade (Mage = 15.87±0.6; 64 [51.6%] female). 103 additional substance use self-report data points were collected at wave 5 (Mage = 17.0±0.6; 54 [52.4%] female) with 11 (10.7%) in 9th grade, 50 (48.5%) in 10th grade, and 42 (40.8%) in 11th grade. Of the 170 individuals included in the final sample, 85 had 6th grade fMRI data (Mage = 12.6±0.4; 55.3% female), 143 had 7th grade fMRI data (Mage = 13.4±0.5; 52.4% female), 109 had 8th grade fMRI data (Mage = 14.3±0.5; 47.3% female), and 51 had 9th grade fMRI data (Mage = 15.2±0.4; 47.1% female). Regarding self-reported substance use behavior, 85 had 6th grade data, 140 had 7th grade data, 124 had 8th grade data, 122 had 9th grade data, 100 had 10th grade data, and 42 had 11th grade data.

The majority of primary caregivers were adolescent's biological mothers (82%), but also included biological fathers (10%), other maternal figures (i.e., aunt, grandmother; 6%), and other paternal figures (i.e., stepfather, grandfather; 2%). Thirty-two caregivers completed all questionnaires in Spanish, which were translated and back translated by paid bilingual translators. All adolescent participants were proficient in and completed all materials in English.

MRI data acquisition and analysis. MRI data were collected on a Siemens Prisma MRI, 3-Tesla scanner. For the two functional social incentive delay (SID) task runs, 37 slices (3 mm thick; voxel size = 2.5 x 2.5 x 3 mm) were obtained using a T2*-weighted, single-shot, gradient-echo, echo-planar imaging (EPI) sequence sensitive to blood oxygenation level-dependent (BOLD) effects (195 volumes/run, repetition time [TR] = 2000 ms, echo time [TE] = 25 ms, field of view = 230 mm, 92 × 92 matrix). The orientation for the EPI scans was oblique axial to maximize brain coverage and to reduce noise. T1-weighted structural images were obtained in the sagittal plane using a magnetization-prepared rapid gradient-echo (MPRAGE) sequence (TR = 2400 ms; TE = 2.22 ms; 208 slices; voxel size = 0.8mm^3).

Neuroimaging data preprocessing was performed with *FMRIPrep* v1.5.3 (3) a Nipype-based tool v1.3.1 (4,5) (RRID:SCR_002502) often employing Nilearn (6). T1-weighted structural volumes were corrected for intensity non-uniformity (N4BiasFieldCorrection v2.1.0)(7) and skull-stripped (antsBrainExtraction.sh v2.2.0), using OASIS30ANTs as the target template. Nonlinear registration (ANTs v2.2.0) was performed to spatially normalize T1-weighted volumes to the *ICBM 152 Nonlinear Asymmetrical template version 2009c* (TemplateFlow ID: MNI152NLin2009cAsym (8) (RRID:SCR_008796).

For the functional data, the following preprocessing steps were performed. First, a reference volume and its skull-stripped version were generated using a custom methodology of *fMRIPrep*. This BOLD reference volume was then co-registered to the T1-weighted reference using `bbregister` (FreeSurfer) which implements boundary-based registration (9). Co-registration was configured with six degrees of freedom. Head-motion parameters with respect to the BOLD reference (transformation matrices, and six corresponding rotation and translation parameters) are estimated using `mcflirt` (FSL 5.0.9) (10). BOLD runs were slice-time corrected using `3dTshift` from AFNI 20160207 (11) (RRID:SCR_005927). The BOLD time-series (including slice-timing correction) were resampled onto their original, native space by applying the transforms to correct for head-motion. Gridded (volumetric) resamplings were performed using `antsApplyTransforms` (ANTs), configured with Lanczos single interpolation composing all the pertinent transformations (i.e. head-motion transform matrices, and co-registrations to anatomical and template spaces) to minimize the smoothing effects of other kernels (12). Framewise displacement (FD) was calculated for each functional run, (*Nipype*) (13) and head-motion estimates, calculated in the correction step, and their temporal derivatives (14) were saved. To adjust for the shifted sampling of functional data to the middle of each TR following slice-timing correction , TR/2 was subtracted from event onset times (15) used in participant-level general linear models.

Following preprocessing, timeseries from the two SID runs were scaled to the voxel-wise mean (3dcalc) thereby allowing regression (β) coefficients, calculated per regressor and participant, to be interpreted as an approximation of percent BOLD signal change (% BOLD Δ) from the implicit baseline (57). Functional volumes with FD greater than 0.9 mm were censored (1.2 ± 3.2% of TRs). One participant was excluded due to more than 25% of the TRs in the second SID run being censored. Spatial smoothing was not performed to avoid automatic partial voluming of the HB and VS regions of interest (ROIs) with surrounding tissue and CSF.

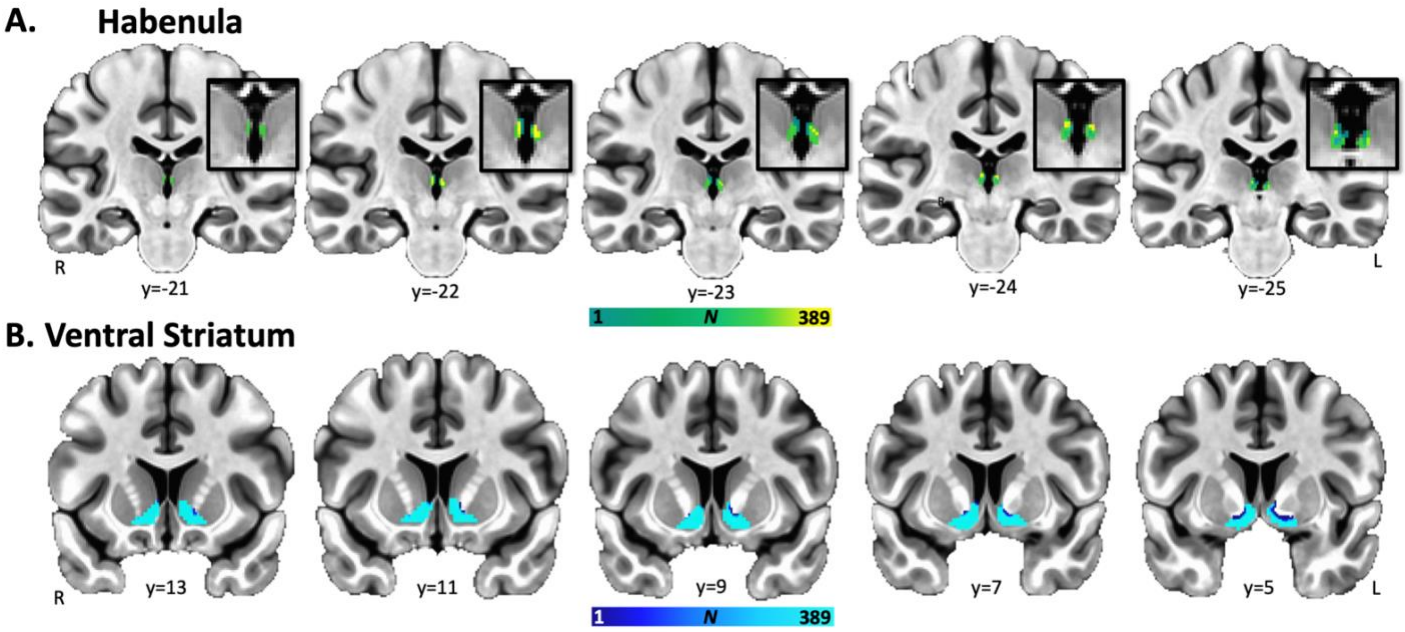
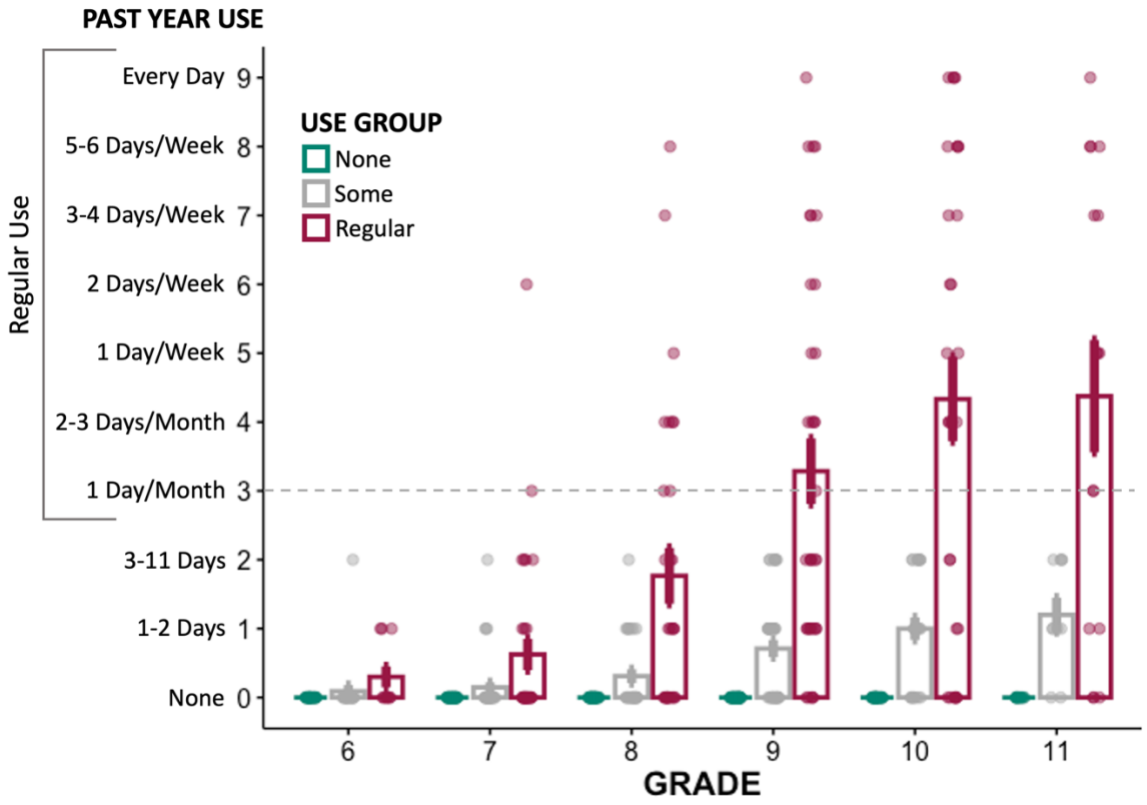


Figure S1. Participant and session specific habenula and ventral striatum masks. Participant/session specific masks were summed to produce an overlap image with voxel values ranging from 0 to 389 (total number of fMRI timepoints) that represent the distribution of the **(A)** habenula (HB) and **(B)** ventral striatum (VS) mask sizes across participants and sessions. Participant and session specific HB and VS masks were defined by subtracting voxels that were outside each participant's and session's anatomically derived gray-matter (GM) mask such that region of interest (ROI) β coefficients were only being extracted from voxels in GM and not from those in cerebral spinal fluid (CSF) or white-matter (WM). Across all participants and sessions, an average of 6.6 ± 2.8 voxels were removed from the bilateral HB mask and an average of 21.9 ± 15.4 voxels were removed from the bilateral VS mask resulting in the HB masks being 7.4 ± 2.8 voxels on average and the VS masks being 331.1 ± 15.4 voxels on average. Across participants and timepoints, ROI size was not correlated with the ROI's β coefficient (p>0.2).



<u>Figure S2</u>. **Longitudinal substance use increases across adolescence by use group**. Adolescent past year substance use at each timepoint. At each wave of data collection, adolescent participants reported how many days in the last year they vaped electronic-cigarettes, smoked cigarettes, had at least one drink of alcohol, smoked marijuana, used prescription substances they did not have a prescription for, and used other substances including crystal meth, cocaine, heroin, ecstasy, LSD, or PCP. Use was reported using the following scale (0)=days in the past year, (1)=1 to 2 days in the past year, (2)=3 to 11 days in the past year, (3)=1 day a month, (4)=2 to 3 days a month, (5)=1 day a week, (6)=2 days a week, (7)=3 to 4 days a week, (8)=5 to 6 days a week, (9)=Every day. Each subject's maximum yearly use of any substance over all grades was used to determine their substance group. Regular substance use was considered using drugs at least once per month for the past year while some substance use was considered using drugs at least once in the past year, but less than once per month. Use group differences in substance use at each grade were assessed with a Kruskal-Wallis test for ordinal variables which is similar to a one-way ANOVA but ranks are used rather than actual data points. As expected, at each grade, substance use groups' past year use significantly differed (p's<0.001).

<u>Substance use across 6th-11th grade</u>. Each subject's maximum yearly use of any substance over all grades was used to determine their substance group. Across all timepoints, 50.3% of adolescents reported never using any substances (no use), 27.2% reported using substances 1-11 days a year (some use), and 22.5% reported using substances at least once a month for a year (regular use; **Figure S3C**). The percent of participants reporting some and regular substance use increased longitudinally (**Figure S3A**) with 2.1% reporting regular use in 7th grade (n=140), 8.9% reporting

regular use in 8th grade (*n*=124), 13.1% reporting regular use in 9th grade (*n*=122), 20.0% reporting regular use in 10th grade (*n*=100), and 28.6% reporting regular use in 11th grade (*n*=42). None of the adolescent participants in our sample reported any regular substance use in 6th grade (*n*=85). A breakdown of the types of substances used by grade is displayed in **Figure S3B** and largely corresponded with national estimates (16,17). Alcohol drinking was the most commonly used in 6th grade with 4.7% of participants reporting some use. Whereas 3.5% reported some cigarette smoking, 2.4% reported some vaping, and no one reported any marijuana, prescription drug, or other substance use. In 7th grade, drinking alcohol and vaping were the most common regularly used substances with 1.4% reporting regular drinking and 1.4% reporting regular vaping. In 8th grade vaping was the most common regularly used substance with 4.8% reporting regular vaping in the past year, followed by drinking, with 3.2% reporting regular drinking in the past year and, marijuana use with 2.4% reporting regular use in the last year. In 9th grade marijuana and vaping were the most common regularly used substances with 8.2% reporting regular marijuana use 9.0% reporting regular vaping, following by drinking with 8.0% reporting regular drinking. In 10th grade, marijuana was the most common regularly used substance with 14.0% reporting regular use, followed by vaping with 11.0% reporting regular use, and drinking with 4.8% reporting regular drinking. Finally, in 11th grade, 21.4% reported regular vaping (similar to national estimates among highschoolers), while no one reported regular cigarette smoking. 16.7% reported regular marijuana use, 11.9% reported regular alcohol drinking, and no one reported regular prescription drug or other drug use.

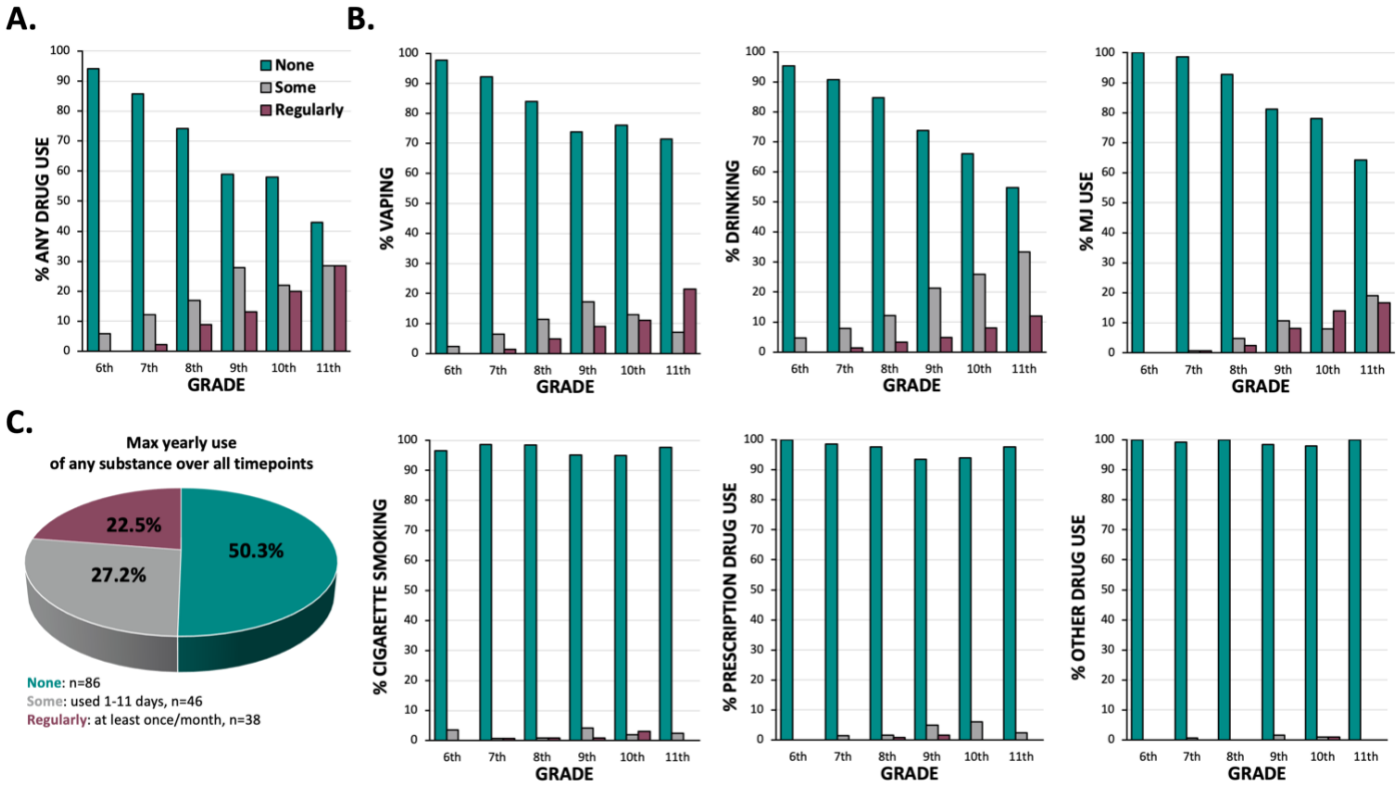


Figure S3. Substance use by grade. (A) Percent of participants in each grade reporting none, some, or regular use of any substance in the past year (**None**: teal; no reported use in the last year, **Some**: gray; use of any substance on 1 -11 days in the last year; **Regularly**: pink; use of any substance at least once a month or more in the last year). The percent of participants reporting some and regular substance use increased longitudinally with no participants reporting regular use in 6th grade (*n*=85), 2.1% reporting regular use in 7th grade (*n*=140), 8.9% reporting regular use in 8th grade (*n*=124), 13.1% reporting regular use in 9th grade (*n*=122), 20.0% reporting regular use in 10th grade (*n*=100), and 28.6% reporting regular use in 11th grade (*n*=42). **(B)** Percent of participants in each grade reporting none, some, or regular use of each substance type. In 6th grade, no participants reported regular use of any substance use. Alcohol drinking was the most commonly used in 6th grade with 4.7% of participants reporting some use. Whereas 3.5% reported some cigarette smoking, 2.4% reported some vaping, and no one reported any marijuana, prescription drug, or other substance use. In 7th grade, drinking alcohol and vaping were the most common regularly used substances with 1.4% reporting regular drinking and 1.4% reporting regular vaping. In 8th grade vaping was the most common regularly used substance with 4.8% reporting regular vaping in the past year, followed by drinking, with 3.2% reporting regular drinking in the past year and, marijuana use with 2.4% reporting regular use in the last year. In 9th grade marijuana and vaping were the most common regularly used substances with 8.2% reporting regular marijuana use 9.0% reporting regular vaping, following by drinking with 4.9% reporting regular drinking. In 10th grade, marijuana was the most common regularly used substance with 14.0% reporting regular use, followed by vaping, with 11.0% reporting regular use and drinking with 8.0% reporting regular drinking. Finally, in 11th grade, 21.4% reported regular vaping (similar to national estimates among highschoolers), while no one reported regular cigarette smoking. 16.7% reported regular marijuana use, 11.9% reported regular alcohol drinking, and no one reported regular prescription drug or other drug use. **(C)** Percent of sample in each group substance. Each subject's maximum yearly use of any substance over all grades was used to determine their substance group.

Whole-brain responsivity to social reinforcement cues and outcomes. To assess habenular responsivity to social reinforcement in the social incentive delay (SID) task, we conducted an exploratory whole-brain *t*-test for both contrasts of interest (3dttest++, $p_{\text{voxel-level}}<0.0001$, $p_{\text{cluster-level}}<0.05$, cluster extent threshold = 8 voxels for all contrasts, determined via ACF 3dclustsim) within a sample-specific gray-matter mask (183,714 voxels). Specifically, each subject's whole brain maps were averaged across all available timepoints and were entered into a whole-brain *t*-test across all subjects. We observed increased activity in small clusters overlapping with habenular nuclei in response to social reward hit outcomes vs. social reward miss outcomes **(Figure S4A).** Whereas in contrast, we observed decreased activity in habenular nuclei in response to social punishment hit outcomes vs. social punishment miss outcomes **(Figure S4B)**. Whereas prior work has indicated increased HB activity associated with negative outcomes, we observed significantly increased HB activity in response to both positive and negative social outcomes compared to neutral outcomes at the whole-brain level. This observation may reflect limitations of the BOLD signal. Specifically, prior preclinical research indicates that most neurons in lateral HB respond to both aversion and reward stimuli (18). While this work demonstrated that the majority of neurons in lateral HB were aversion-activated and reward-inhibited, BOLD signal would not be able to distinguish these two functions and would therefore merely be able to indicate locations of recent energy expenditure. Nonetheless, these exploratory whole brain results suggest that the SID

differentially elicits adolescent HB function similar to other feedback processing tasks (19,20). In response to social reward outcomes (smiling face) vs. reward omission (non-informative face), we also observed increased activity in the bilateral striatum, ventral medial prefrontal cortex (vmPFC), bilateral fusiform gyrus, bilateral middle temporal gyrus, bilateral amygdala, bilateral inferior frontal gyrus in addition to clusters in the supplementary motor area, precentral gyrus and postcentral gyrus. Similarly, in response to social punishment avoidance (non-informative face) vs. social punishment outcomes (scowling face) we observed increased bilateral striatal activity and increased activity in the supplementary motor area, precentral gyrus, and postcentral gyrus. Additionally, increased activity was also observed in bilateral clusters in the middle prefrontal gyrus and superior parietal lobe. Increased activity in large bilateral occipital clusters was additionally observed for both task contrasts. Our findings largely correspond to activity reported in a recent meta-analysis of SID task fMRI studies (21)(22). Cluster coordinates are reported in **Table S1**.

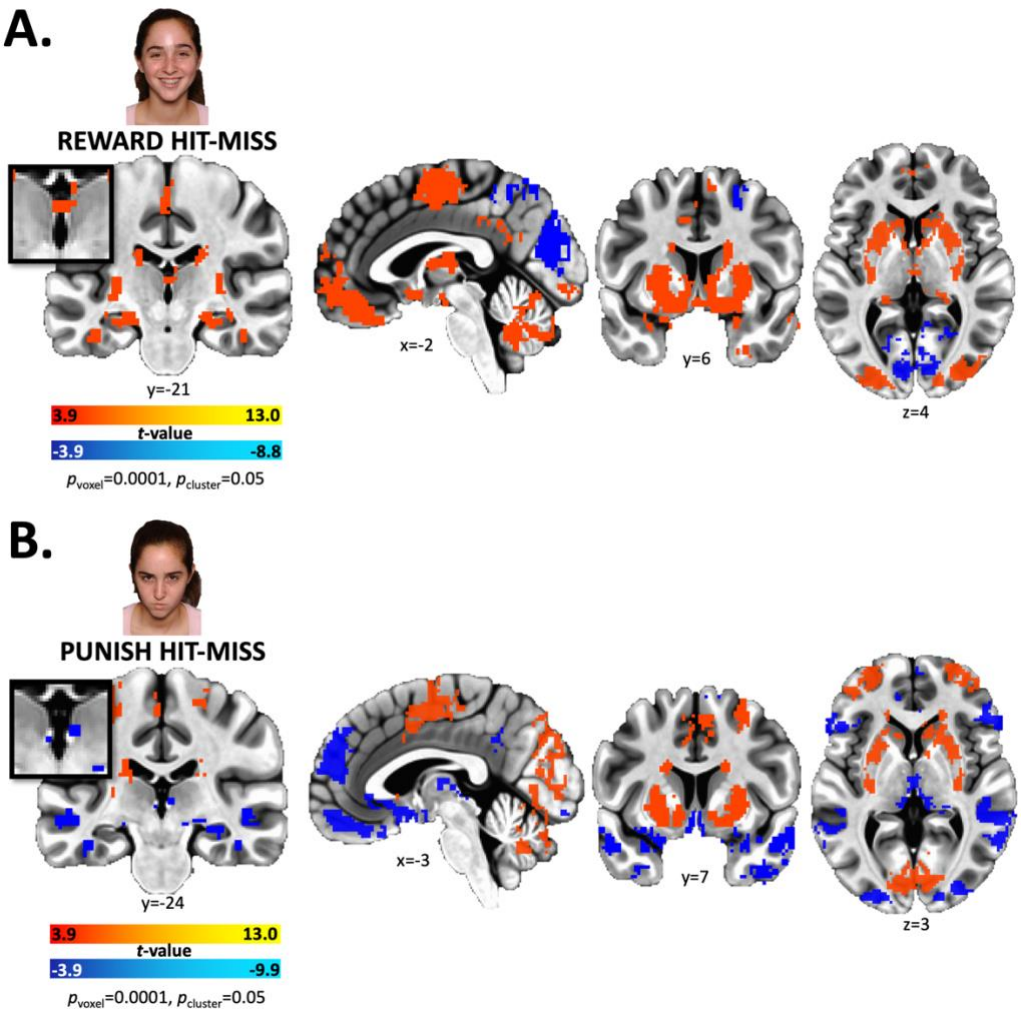


Figure S4. **Exploratory whole brain social reinforcement responsivity**. Exploratory whole brain contrast images were calculated to assess habenular responsivity to social reinforcement in the social incentive delay (SID) task. For each subject, whole-brain Reward hit vs. Reward miss [Rhit-Rmiss] and Punishment hit vs. Punishment miss [Phit-Pmiss] contrast images were averaged

across all their available timepoints (3dcalc) such that each subject had a mean whole-brain map for each of the two task contrasts of interest. All subjects' mean images were then entered into a whole-brain t-test for each contrast (3dttest++, $p_{\text{voxel-corrected}}<0.0001$, $p_{\text{cluster-corrected}}<0.05$, cluster extent threshold = 8 voxels for all contrasts, determined via ACF 3dclustsim). Whole-brain analyses were run within a gray matter mask (183,714 voxels) calculated as voxels in which 70% of the sample's gray matter parcellation overlapped. We then subtracted the 70% overlap sample cerebral spinal fluid parcellation from this mask. **(A)** In response to social reward outcomes (smiling face) vs. reward omission (non-informative face), we observed increased activity in small clusters overlapping with habenular nuclei and increased activity in the bilateral striatum, ventral medial prefrontal cortex (vmPFC), bilateral fusiform gyrus, bilateral middle temporal gyrus, bilateral amygdala, bilateral inferior frontal gyrus in addition to clusters in the supplementary motor area, precentral gyrus and postcentral gyrus. **(B)** In response to social punishment avoidance (non-informative face) vs. social punishment outcomes (scowling face) we observed decreased activity in habenular nuclei and similar to the reward outcomes, observed increased bilateral striatal activity and increased activity in the supplementary motor area, precentral gyrus, and postcentral gyrus. Additionally, increased activity was also observed in bilateral clusters in the middle prefrontal gyrus and superior parietal lobe.

Table S1. Exploratory whole brain social reinforcement responsivity. For each subject, whole-brain Reward hit vs. Reward miss [Rhit-Rmiss], and Punishment hit vs. Punishment miss [Phit-Pmiss] contrast images were averaged across all their available timepoints (3dcalc) such that each subject had a mean whole-brain map for each task contrasts of interest. All subjects' mean images (n=169) were then entered into a whole-brain t-test for each contrast (3dttest++, $p_{\text{voxel-level}}<0.0001$, $p_{\text{cluster-level}}<0.05$, cluster extent threshold = 8 voxels for all contrasts, determined via ACF 3dclustsim). Only clusters over 50 voxels are reported in the table.

	Region	Hemisphere	Peak Coordinates (MNI, LPI)			Cluster Size (# of Voxels)
			X	Y	Z	
Reward Hit - Reward Miss (Rhit > Rmiss)						
1	**Habenula**/thalamus/striatum/amygdala/occipital gyrus/fusiform gyrus	B	-16	-2	-15	12462
2	**Ventral medial frontal** gyrus	B	2	-64	-7	1371
3	**Precentral** gyrus	L	-40	-24	67	700
4	**Medial frontal** gyrus	B	0	-6	47	582
5	**Inferior frontal** gyrus (p. Orbitalis)	R	36	36	-23	287
6	**Posterior cingulate** (BA 23)	B	0	-48	21	281
7	**Inferior frontal** gyrus (p. Orbitalis)	L	-38	26	-21	271
8	**Middle temporal** gyrus (BA 21)	R	64	0	-13	265
9	**Precentral** gyrus	R	22	-26	59	190
10	**Middle temporal** gyrus (BA 21)	L	-64	-4	-9	112
11	**Medial temporal** pole	R	42	24	-39	111
12	**Dorsal lateral frontal** gyrus (BA 6)	R	38	-12	47	86
13	**Superior temporal** gyrus	R	46	-36	3	70
14	**Cerebellar** tonsil	R	16	-36	-45	61

15	**Cingulate** gyrus	B	-4	-2	29	54
Reward Miss - Reward Hit (Rmiss > Rhit)						
1	**Cuneus**/precuneus	B	2	-64	59	3679
2	**Middle frontal** gyrus (BA 6)	R	26	8	67	184
3	**Inferior parietal** gyrus (BA 40)	R	58	-42	49	139
4	**Middle frontal** gyrus (BA 6)	L	-28	10	65	99
5	**Cuneus**	R	14	-88	27	65
Punishment Hit - Punishment Miss (Phit > Pmiss)						
1	**Occipital**/superior parietal gyrus	B	-44	-54	59	8233
2	**Caudate**/nucleus accumbens/putamen	R	2	-12	13	1530
3	**Caudate**/nucleus accumbens/putamen	L	-26	0	-9	1268
4	**Supplementary motor area** (BA 6)	B	-2	-12	71	990
5	**Precentral**/postcentral gyrus	L	-40	-24	67	712
6	**Superior frontal** gyrus (BA 10)	R	30	66	-1	602
7	**Middle frontal** gyrus (BA 6)	R	32	4	67	478
8	**Middle frontal** gyrus (BA 6)	L	-44	52	11	414
9	**Middle frontal** gyrus (BA 9)	R	42	36	39	249
10	**Precentral** gyrus (BA 4)	R	20	-30	61	120
11	**Middle frontal** gyrus	L	-34	-4	67	92
12	**Superior temporal** gyrus	L	-54	0	1	82
13	**Posterior cingulate** (BA 23)	B	2	-30	27	65
14	**Anterior insula**/inferior frontal gyrus	R	32	24	-7	51
Punishment Miss - Punishment Hit (Pmiss > Phit)						
1	**Occipital**/fusiform/inferior frontal gyrus	R	44	-52	-25	6565
2	**Occipital**/fusiform/inferior frontal gyrus	L	-20	-90	-19	4125
3	**Medial frontal** gyrus (BA 9)	B	-2	58	27	1071
4	**Ventral medial frontal**/orbital gyrus (BA 25)	B	-2	6	-11	769
5	**Superior temporal** gyrus	L	-68	-52	9	719
6	**Habenula**/parahippocampal gyrus	B	14	-32	-1	340
7	**Posterior cingulate** (BA 23)	R	-2	-48	23	250
8	**Superior frontal** gyrus	R	12	18	67	164
9	**Middle occipital** gyrus	R	26	-90	7	159

NOTE. Voxel size: 2 x 2 x 2 mm^3. X: Left (-), Right (+); Y: Posterior (-), Anterior (+); Z: Inferior (-), Superior (+). Region labels informed by the AFNI Talairach daemon atlas. See Supplemental **Figure S1** for graphical representation.

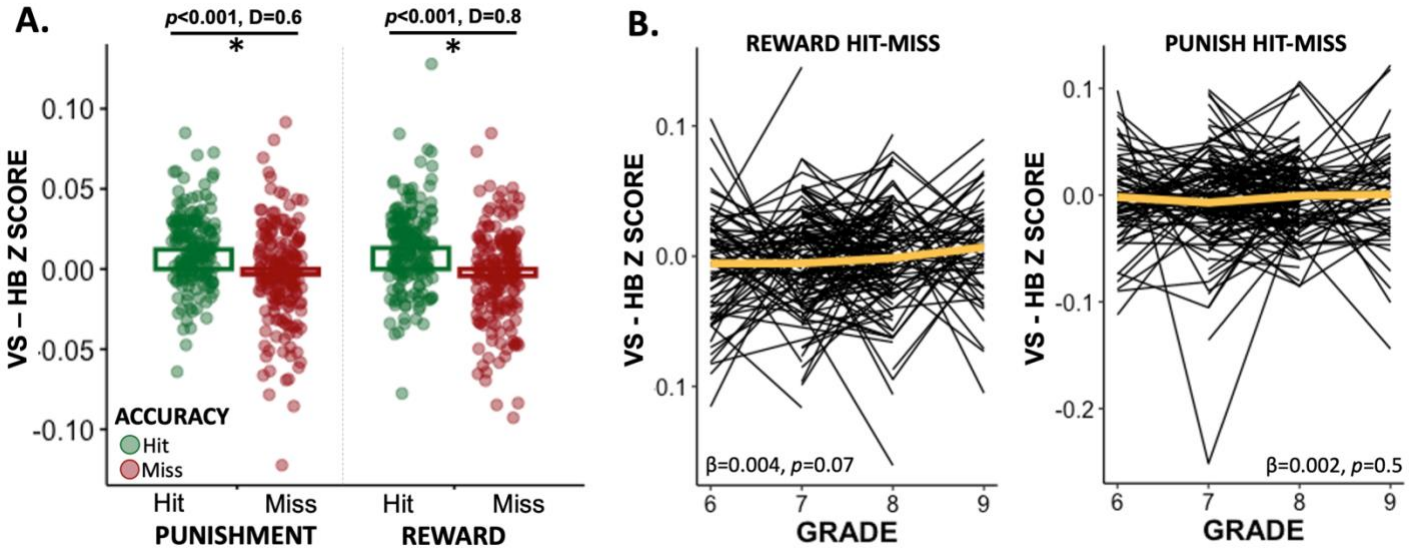
Psychophysiological interaction (PPI) analysis. To examine task-dependent VS and HB correlations we conducted an exploratory generalized psychophysiological interaction (PPI) analysis using AFNI (v.22), following the approach described in prior research (23,24). We extracted the average timeseries for each participant's bilateral VS ROI for both SID runs. Timeseries were detrended and deconvolved with the hemodynamic response (Gamma) function. To generate four VS × task event interaction regressors, we multiplied each task event of interest

(i.e., Rhit, Rmiss, Phit, Pmiss) by the deconvolved VS signal. These four new regressors as well as the VS timeseries were added to the original participant-level general linear model. Whole brain task-event dependent VS correlation coefficient maps were converted to *Z*-score maps and *Z*-scores were averaged across voxels in each participant's bilateral HB ROI.

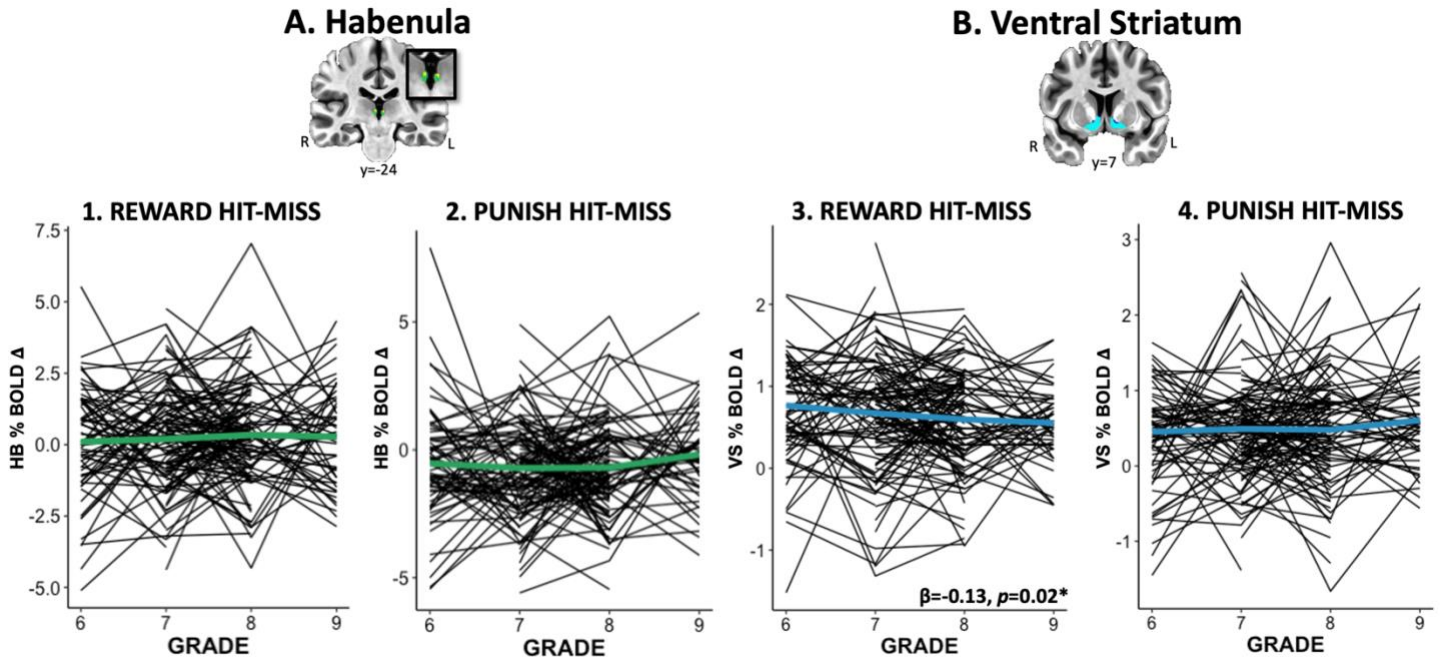
We conducted an exploratory two-way, 2(TRIAL TYPE: reward vs. punishment) × 2(ACCURACY: hit vs. miss) repeated-measures ANOVA on task-event dependent VS-HB correlations averaged across each participant's available timepoints (**Figure S5A**). We observed a significant main effect of ACCURACY ($F[1, 168]$=88.2, p<0.001, η_p^2 = 0.34). Follow-up *t*-tests confirmed that, for both punishment (p<0.001) and reward trials (p<0.001), VS activity was significantly more negatively correlated with HB activity when receiving negative feedback for inaccurate responses (misses) than when receiving positive feedback for accurate responses (Hits). This result is congruent with our finding of increased VS responsivity, but decreased HB responsivity, to hit vs. miss outcomes on punishment trails. Taken together these observations may reflect the HB's role in regulating activity in other brain regions in response to negative outcomes (25,26).

To examine developmental changes in task dependent VS and HB correlations, we assessed the effect of grade on task contrast (Rhit-Rmiss and Phit-Pmiss) dependent VS-HB *Z*-scores within linear mixed-effect models (lmer, R-package). The random intercept and slope of grade as well as their correlation were modeled. Across the sample, we did not observe significant changes in VS-HB correlations for reward hit vs. reward miss or punishment hit vs. punishment miss task contrasts (p'*s*>0.07; **Figure S5B**). However, we note a marginal longitudinally increasing trend of the Rhit-Rmiss dependent VS-HB correlation (p=0.07).

Finally, we assessed whether developmental changes in VS-HB task-dependent correlations, across 6th to 11th grade, differed based on participants' maximum reported yearly substance use. We conducted exploratory USE×GRADE interactions on Rhit-Rmiss and Phit-Pmiss task-contrast-dependent VS-HB correlations, again within restricted maximum likelihood estimation (REML) linear mixed-effect models, allowing for random intercepts and slopes of grade as well as their correlation across subjects. We did not observe significant drug use by grade interactions or drug use main effects for the VS's Rhit-Rmiss-dependent correlation (p's>0.6) with the HB nor its Phit-Pmiss-dependent correlation with the HB (p's>0.2).



<u>Figure S5</u>. Ventral Striatum and habenula physio-psychological interaction and grade effects. <u>(A)</u> ACCURACY main effect for VS-HB task-dependent correlations ($F[1, 168]$=88.2, p<0.001, η_p^2 = 0.34). For both punishment and reward trials, VS activity was significantly more negatively correlated with HB activity when receiving negative feedback for inaccurate responses (Misses) than when receiving positive feedback for accurate responses (Hits). **<u>(B)</u>** No significant grade-related changes in VS-HB correlations for reward hit vs. reward miss or punishment hit vs. punishment miss task contrasts were observed. Although we note a non-significant (p=0.07) longitudinally increasing trend of the reward hit-miss contrast-dependent VS-HB correlation that corresponds with our observation of longitudinally decreasing reward hit-miss VS responsivity.



<u>Figure S6</u>. Responsivity to social reinforcement across 6th- 9th grade. To examine developmental changes in responsivity to social reinforcement, we assessed the effect of grade on habenula (HB) and ventral striatum (VS) task contrast β coefficients within linear mixed-effect models (lmer, R-package). Given the interest in within-person reliability of task-based brain activity across multiple developmental timepoints (27), we separately interrogated within- and between-person variability in HB and VS SID activity as well as the within- and between-person effects of grade. To disentangle these effects, both grand-mean centered, and participant-mean centered grade variables were entered as predictors. The random intercept and slope of grade as well as their correlation were modeled within subject. **<u>(A)</u>** Across the full sample, there was more between-adolescent variance than within-adolescent variance in grade effects on Rhit-Rmiss and Phit-Pmiss HB activity. No significant within-person effects of grade were observed on (1) Rhit-Rmiss or (2) Phit-Pmiss Habenula (HB) activity (*p*'s>0.8). **<u>(B)</u>** In contrast Rhit-Rmiss VS displayed higher a within-person effect of grade (-0.13) than between-person effect (-0.04) such that Rhit-Rmiss ventral striatal activity displayed significant within-person longitudinal decreases (β = -0.13 ± 0.06, *t*[*191.4*] = -2.3, *p* = 0.022). Phit-Pmiss ventral striatal activity displayed more between-adolescent variance than within-adolescent variance in grade effects and did not significantly change across grade (*p*=0.8). Regarding within-person reliability of task-based brain activity, in all instances we observed more within-person variably than between-person variability which supports Frohner et al., 2019 findings suggesting nontrivial within-adolescent variability in task-based brain activity across time (27). Specifically, Rhit-Rmiss HB activity displayed 2.9±1.7 within-person variance and 0.25±0.5 between-person variance whereas Phit-Pmiss HB activity displayed 3.47±1.9 within-person variance and 0.09±0.3 between-person variance. Approximately 7.9% of the total variability in Rhit-Rmiss HB activity is due to between-person variability and, on average, Rhit-Rmiss HB activity is correlated 0.079 across timepoints, within any one adolescent. Approximately 2.4% of the total variability in Phit-Pmiss HB activity is due to between-person variability and, on average, Phit-Pmiss HB activity is correlated 0.024 across timepoints, within any one adolescent. VS activity displayed more within-person stability than the

HB with approximately 19.4% of the total variability in Rhit-Rmiss VS activity is due to between-person differences. On average Rhit-Rmiss VS activity is correlated 0.19 across timepoints, within any one adolescent. Approximately 11.2% of the total variability in Phit-Pmiss VS activity is due to between-person differences and, on average, Phit-Pmiss VS activity is correlated 0.11 across timepoints, within any one adolescent. Rhit-Rmiss VS activity displayed 0.33±0.6 within-person variance and 0.08±0.3 between-person variance whereas Phit-Pmiss VS activity displayed 0.41±0.6 within-person variance and 0.05±0.2 between-person variance. Implications of these dispirit sources of variability should be considered when interpreting developmental changes in SID-related brain function. However, primary analyses of the current manuscript focus on explaining between-person variability in longitudinal grade effects due to drug use.

SUPPLEMENTAL REFERENCES

1. Jorgensen NA, Muscatell KA, McCormick EM, Prinstein MJ, Lindquist KA, Telzer EH. Neighborhood Disadvantage, Race/Ethnicity, and Neural Sensitivity to Social Threat and Reward among Adolescents. Soc Cogn Affect Neurosci. 2022 Sep 30;nsac053.

2. Telzer EH, Jorgensen NA, Prinstein MJ, Lindquist KA. Neurobiological Sensitivity to Social Rewards and Punishments Moderates Link Between Peer Norms and Adolescent Risk Taking. Child Dev. 2021;92(2):731–45.

3. Esteban O, Markiewicz CJ, Blair RW, Moodie CA, Isik AI, Erramuzpe A, et al. fMRIPrep: a robust preprocessing pipeline for functional MRI. Nat Methods. 2019 Jan;16(1):111–6.

4. Gorgolewski K, Burns C, Madison C, Clark D, Halchenko Y, Waskom M, et al. Nipype: A Flexible, Lightweight and Extensible Neuroimaging Data Processing Framework in Python. Front Neuroinformatics [Internet]. 2011 [cited 2022 Aug 8];5. Available from: https://www.frontiersin.org/articles/10.3389/fninf.2011.00013

5. Esteban O, Markiewicz CJ, Burns C, Goncalves M, Jarecka D, Ziegler E, et al. nipy/nipype: 1.8.3 [Internet]. Zenodo; 2022 [cited 2022 Aug 8]. Available from: https://zenodo.org/record/6834519

6. Abraham A, Pedregosa F, Eickenberg M, Gervais P, Mueller A, Kossaifi J, et al. Machine learning for neuroimaging with scikit-learn. Front Neuroinformatics [Internet]. 2014 [cited 2022 Aug 8];8. Available from: https://www.frontiersin.org/articles/10.3389/fninf.2014.00014

7. Tustison NJ, Avants BB, Cook PA, Zheng Y, Egan A, Yushkevich PA, et al. N4ITK: Improved N3 Bias Correction. IEEE Trans Med Imaging. 2010 Jun;29(6):1310–20.

8. Fonov V, Evans A, McKinstry R, Almli C, Collins D. Unbiased nonlinear average age-appropriate brain templates from birth to adulthood. NeuroImage. 2009 Jul 1;47:S102.

9. Greve DN, Fischl B. Accurate and robust brain image alignment using boundary-based registration. NeuroImage. 2009 Oct 15;48(1):63–72.

10. Jenkinson M, Bannister P, Brady M, Smith S. Improved Optimization for the Robust and Accurate Linear Registration and Motion Correction of Brain Images. NeuroImage. 2002 Oct 1;17(2):825–41.

11. Cox RW, Hyde JS. Software tools for analysis and visualization of fMRI data. NMR Biomed. 1997;10(4–5):171–8.

12. Lanczos C. Evaluation of Noisy Data. J Soc Ind Appl Math Ser B Numer Anal. 1964 Jan;1(1):76–85.

13. Power JD, Mitra A, Laumann TO, Snyder AZ, Schlaggar BL, Petersen SE. Methods to detect, characterize, and remove motion artifact in resting state fMRI. NeuroImage. 2014 Jan 1;84:320–41.

14. Satterthwaite TD, Elliott MA, Gerraty RT, Ruparel K, Loughead J, Calkins ME, et al. An improved framework for confound regression and filtering for control of motion artifact in the preprocessing of resting-state functional connectivity data. NeuroImage. 2013 Jan 1;64:240–56.

15. Russell P, Jeanette M. Slice timing correction in fMRIprep and linear modeling [Internet]. Stanford Center for Reproducable Neuroscience. https://reproducibility.stanford.edu/slice-timing-correction-in-fmriprep-and-linear-modeling/. Available from: https://reproducibility.stanford.edu/slice-timing-correction-in-fmriprep-and-linear-modeling/

16. Creamer MR, Everett Jones S, Gentzke AS, Jamal A, King BA. Tobacco Product Use Among High School Students — Youth Risk Behavior Survey, United States, 2019. MMWR Suppl. 2020 Aug 21;69(1):56–63.

17. Jones CM, Clayton HB, Deputy NP, Roehler DR, Ko JY, Esser MB, et al. Prescription Opioid Misuse and Use of Alcohol and Other Substances Among High School Students — Youth Risk Behavior Survey, United States, 2019. MMWR Suppl. 2020 Aug 21;69(1):38–46.

18. Li J, Fan R, Liu X, Shen X, Liu X, Zhao H. The convergence of aversion and reward signals in individual neurons of the mice lateral habenula. Exp Neurol. 2021 May 1;339:113637.

19. Ullsperger M, Cramon DY von. Error Monitoring Using External Feedback: Specific Roles of the Habenular Complex, the Reward System, and the Cingulate Motor Area Revealed by Functional Magnetic Resonance Imaging. J Neurosci. 2003 May 15;23(10):4308–14.

20. Flannery JS, Riedel MC, Poudel R, Laird AR, Ross TJ, Salmeron BJ, et al. Habenular and striatal activity during performance feedback are differentially linked with state-like and trait-like aspects of tobacco use disorder. Sci Adv [Internet]. 2019 Oct [cited 2021 Dec 13];5(10). Available from: https://www.ncbi.nlm.nih.gov/labs/pmc/articles/PMC6785263/

21. Martins D, Rademacher L, Gabay AS, Taylor R, Richey JA, Smith DV, et al. Mapping social reward and punishment processing in the human brain: A voxel-based meta-analysis of

neuroimaging findings using the social incentive delay task. Neurosci Biobehav Rev. 2021 Mar 1;122:1–17.

22. Kohls G, Perino MT, Taylor JM, Madva EN, Cayless SJ, Troiani V, et al. The nucleus accumbens is involved in both the pursuit of social reward and the avoidance of social punishment. Neuropsychologia. 2013 Sep 1;51(11):2062–9.

23. McLaren DG, Ries ML, Xu G, Johnson SC. A Generalized Form of Context-Dependent Psychophysiological Interactions (gPPI): A Comparison to Standard Approaches. Neuroimage. 2012 Jul 16;61(4):1277–86.

24. Cisler JM, Bush K, Steele JS. A comparison of statistical methods for detecting context-modulated functional connectivity in fMRI. NeuroImage. 2014 Jan 1;84:1042–52.

25. Baker PM, Mizumori SJY. Control of behavioral flexibility by the lateral habenula. Pharmacol Biochem Behav. 2017 Nov 1;162:62–8.

26. Kawai T, Yamada H, Sato N, Takada M, Matsumoto M. Roles of the Lateral Habenula and Anterior Cingulate Cortex in Negative Outcome Monitoring and Behavioral Adjustment in Nonhuman Primates. Neuron. 2015 Nov 18;88(4):792–804.

27. Fröhner JH, Teckentrup V, Smolka MN, Kroemer NB. Addressing the reliability fallacy in fMRI: Similar group effects may arise from unreliable individual effects. NeuroImage. 2019 Jul 15;195:174–89.