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Age Moderates the Effect of Acute Dopamine Depletion on Passive Avoidance Learning

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Abstract

Despite extensive links between reinforcement-based learning and dopamine (DA), studies to date have not found consistent effects of acute DA reduction on reinforcement learning in both men and women. Here, we tested the effects of reducing DA on reward- and punishment-based learning using the deterministic passive avoidance learning (PAL) task We tested 16 (5 female) adults (ages 22–40) in a randomized, cross-over design to determine whether reducing global DA by administering an amino acid beverage deficient in the DA precursors, phenylalanine and tyrosine (P/T[−]), would affect performance on the PAL task. We found that P/T[−] beverage effects on PAL performance were modulated by age. In particular, we found that P/T depletion significantly improved learning from punishment with increasing participant age. Participants committed 1.49 fewer passive avoidance errors per additional year of age (95% CI, −0.71 – −2.27, r=−0.74, p=0.001). Moreover, in this small sample, P/T depletion improved learning from punishment in adults (ages 26–40) while it impaired learning from punishment in emerging adults (ages 22–25). We observed similar, but non-significant trends in learning from reward. While there was no overall effect of P/T-depletion on reaction time (RT), there was a relationship between the effect of P/T depletion on PAL performance and RT; those who responded more slowly on the P/T[−] beverage also made more errors on the P/T[−] beverage. When P/T-depletion slowed RT after a correct response, there was a worsening of PAL task performance; there was no similar relationship for the RT after an incorrect response and PAL task performance. Moreover, among emerging adults, changes in mood on the P/T[−] beverage negatively correlated with learning from reward on the P/T[−] beverage. Together, we found that both reward- and punishment-based learning are sensitive to central catecholamine levels, and that these effects of acute DA reduction vary with age.

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Keywords

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1. INTRODUCTION

Data from animal models have long established that dopamine (DA) signaling modulates reinforcement learning (Bayer and Glimcher, 2005, Satoh *et al.*, 2003, Schultz, 2002). Links between DA signaling and reinforcement learning have also been established in humans, where changes in striatal DA signaling differentially affect learning from positive and negative feedback (Cools *et al.*, 2006, Cools *et al.*, 2009, Frank, 2005, Frank *et al.*, 2004, Moustafa *et al.*, 2008, Pessiglione *et al.*, 2006, Robinson *et al.*, 2010, Shohamy *et al.*, 2008); however this issue remains incompletely explored. Both reward-based and punishment-based learning are adaptive, and both may depend to some degree on DA signaling. To investigate DA's role in reinforcement learning, we used a passive avoidance learning (PAL) task, which quantifies learning from both positive and negative feedback (Newman and Kosson, 1986), in the context of acute DA precursor depletion in healthy human subjects.

Successful PAL task performance involves a learning period followed by a plateau phase. Neuroimaging data indicate that increased activation with successful PAL task learning, in response to both rewarded or punished stimuli, occurs in the rostral anterior cingulate, insula, caudate, and amygdala, which are all DA terminal fields (Kosson *et al.*, 2006). The role of DA in PAL task performance has been investigated using acute DA-depletion with alpha-methyl-paratyrosine (AMPT), a competitive inhibitor of the rate-limiting enzyme in DA synthesis, in females with major depressive order in full remission and healthy controls (Hasler *et al.*, 2009). All participants were less likely to respond to rewarded stimuli later in the task than in earlier in the task, but there was no effect of AMPT on responding to punished stimuli (Hasler *et al.*, 2009).

To date, no studies in medically healthy males and females have examined the effect of acute DA-depletion on PAL task performance. Therefore, we tested whether reducing DA levels with an amino acid beverage deficient in the amino acids required for DA synthesis, phenylalanine and tyrosine, would affect PAL task performance in both medically healthy males and females. We hypothesized that the phenylalanine/tyrosine-depleted (P/T[−]) beverage would decrease responding to rewarded stimuli and increase responding to punished stimuli compared to the controlled/balanced amino acid beverage. Moreover, the lower age bound for recruitment to this study was set at 22 years based on published data showing that functional brain maturation asymptotes at ~22 years of age (Dosenbach *et al.*, 2010). However, abundant evidence indicates that the emerging adult period, most commonly defined as ages 18–25 (Arnett, 2000), is distinct from adulthood in numerous respects. Although emerging adulthood has been defined largely based on cultural factors, emerging adulthood is also characterized by ongoing neural development in brain regions associated with self-regulation and inhibitory control (Sowell et al., 1999), which play a role in passive avoidance learning. Furthermore, recent data show impaired inhibitory control

within an affective information processing context among emerging adults relative to adults (Cohen-Gilbert *et al.*, 2014). As such, we also separated participants into emerging adult (22–25 years old) and adult (26–40 years old) groups to determine whether the response to dopamine depletion differed among emerging adults.

2. METHODS

2.1 Participants

Participants (*n*=16, 5 females) were recruited from the University of North Carolina at Chapel Hill (UNC) and surrounding community. Participants were 22–40 yr old native English speakers with a high school education or more. Subjects were free from psychoactive medications or illicit drug use and had no current psychiatric or neurological diagnosis. Smokers were also excluded. Females were not breastfeeding or pregnant (confirmed via urine test), and were tested during the follicular phase (d 1–10) of the menstrual cycle. Participants with phenylketonuria were also excluded. Participants gave written, informed consent in accordance with the guidelines of the UNC Office for Human Research Ethics. Participants were paid for their participation; payment did not depend upon performance. Nine additional subjects were tested, but were excluded from all analyses due to a programming error that rendered their data unusable.

2.2 Procedure

In a double-blind, placebo-controlled, within-subjects, counterbalanced design, we used acute P/T depletion to temporarily reduce central DA levels, using a previously described protocol (Kelm and Boettiger, 2013). Subjects consumed a low protein diet (<20 g) for 24h before each session and fasted from midnight until session onset, which occurred between 7–9 A.M. Following the consent procedure and urine screening, participants completed the Profile of Mood States (POMS) (McNair *et al.*, 1971), and we collected a baseline blood sample via finger prick. Participants then consumed an amino acid beverage (balanced/control or P/T[−]). Participants waited 5h in the lab to allow sufficient time for P/T depletion to occur (Sheehan *et al.*, 1996). We then collected a second blood sample, and the participant completed computerized cognitive testing, followed by the POMS. Participants had access to low protein snacks from 1h post-beverage consumption to 1h before the second blood sample collection. Participants were offered a high protein snack at session end. Sessions were separated by ≥72h.

2.3 Amino Acid Beverages

The amino acid mixes were prepared by SHS International (Liverpool, UK). The balanced/control beverage consisted of (in g): L-alanine, 4.1; L-arginine, 3.7; L-cysteine, 2.0; L-glycine, 2.4; L-histidine, 2.4; L-isoleucine, 6; L-leucine, 10.1; L-lysine, 6.7; L-methionine, 2.3; L-phenylalanine, 4.3; L-proline, 9,2; L-serine, 5.2; L-threonine, 4.9; L-tryptophan, 3.0; L-tyrosine, 5.2; and L-valine, 6.7. The P/T[−] beverage had the same composition except that phenylalanine and tyrosine were omitted. Because these amino acid beverages can cause nausea and emesis, individuals weighing <160lb (*n*=6) received a light version of each beverage that was reduced in composition by 20%. Beverages were mixed with cold water

and a cherry-vanilla, grapefruit, or lemon-lime flavor packet from Nutricia (Gaithersburg, MD) in an 8oz sterile cup.

2.4 Behavioral Inventories

Participants completed questionnaires during the waiting period in session one. Demographic information was collected, including age, gender, ethnicity and socioeconomic status (SES). SES was quantified according to (Hollingshead, 1975) using the modification of (Barratt, 2006). Other standard questionnaires included: the Alcohol Use Disorders Identification Test (AUDIT) (Saunders *et al.*, 1993), the Barratt Impulsiveness Scale (BIS) (Barratt, 1994), part I of the Drug Use Screening inventory (DUSI) (Tarter, 1990), the Family Tree Questionnaire (FTQ) (Mann *et al.*, 1985), part I of the Future Time Perspective Inventory (FTPI) (Wallace, 1956), Rotter's Locus of Control Scale (LOC) (Rotter, 1966), the State-Trait Anxiety Inventory (STAI) (Spielberger, 1985), the Beck Depression Inventory (BDI) (Beck and Steer, 1987), and the Anti-social Practices Scale of the Minnesota Multiphasic Personality Inventory 2 (MMPI) (Butcher *et al.*, 1990). Age groups did not differ on any of these measures, excepting age (Table 1).

2.5 Amino Acid Analysis

We analyzed the pre- and post-beverage blood samples to determine the total plasma P/T levels and the ratio of P/T to other large, neutral amino acids (LNAA; tryptophan, valine, isoleucine, and leucine) to confirm P/T depletion by the P/T[−] beverage. This ratio was calculated from the total serum concentrations of P and T divided by the sum of the concentrations of the other LNAA [(P+T)/ΣLNAA]. We used this combined P/T ratio, as it correlates with DA availability within the brain (Montgomery *et al.*, 2003). Using aseptic technique, we used a contact-activated lancet (BD Biosciences, San Jose, CA) to collect 150μL of blood from the finger. Samples were frozen at −20°C prior to analysis. Amino acid analysis was carried out via gas chromatography-mass spectroscopy as described previously (Kelm and Boettiger, 2013).

2.6 Passive Avoidance Learning Task

The PAL task was adapted from (Newman and Kosson, 1986) and implemented in E-Prime 2.0 (Psychology Software Tools, Inc. (PST), Pittsburgh, PA). Subjects were given task instructions and a short practice session prior to testing. Cognitive testing occurred within the bore of a mock MRI scanner (PST). Stimuli were presented on a color LCD screen, which subjects viewed via a head-coil mounted mirror. Participants made manual responses via a keypad. In the task, participants were presented with a series of numerical stimuli to which they either responded with, or withheld, a manual button press. Stimuli included six unique two-digit "good" numbers, and six unique two-digit "bad" numbers. Stimulus sets were counterbalanced for good versus bad designation across subjects to control for any pre-existing positive or negative associations with the number stimuli among participants. Participants completed a unique version of the task during each session. Within session, all twelve numbers were presented once in pseudorandom order during each block; each participant completed ten blocks. Each number was displayed in white Arial font on a black background for up to 3s. Participants learned through trial and error feedback which

numbers were good or bad. Participants began the task with 5000 points, displayed in the bottom right-hand corner of the screen, and point total was updated in each trial. Participants received 400 points when responding to a good number and lost 400 points when responding to a bad number. There was no feedback when a response was withheld. Participants were instructed to accrue as many points as possible. Therefore, following learning, participants should respond to all good numbers, and withhold responding to all bad numbers. Response type (press or omission) and press reaction time (RT) were recorded for all trials.

2.7 Statistical analysis

Performance on the PAL task was assessed by analysis of omission errors (OEs, failures to respond to rewarded stimuli) and passive avoidance errors (PAEs, responses to punished stimuli). For single factor statistical comparisons within and between groups, we used paired and unpaired two-tailed *t*-tests, respectively. For multi-factorial comparisons, we used repeated measures ANOVA. Linear regression was used to determine if any of the demographic information, behavioral inventories, or reaction times correlated with the effect of P/T depletion on OEs and PAEs. All statistical tests were conducted using Excel (Microsoft Corp., Redmond, WA) or SPSS (SPSS Inc., Chicago, IL). Values reported as mean ± SEM, unless otherwise stated. Effect sizes for ANOVA are reported as η^2; effect sizes for *t*-tests are reported as Cohen's *d*.

3. RESULTS

3.1 Effects of P/T depletion on blood concentrations

Repeated measures ANOVA revealed significant two-way interactions between beverage (P/T[−], placebo) and time point (baseline, +5 h) on serum phenylalanine levels ($F_{(1,15)}$=78.6; p<0.001, η^2=0.35), serum tyrosine levels ($F_{(1,15)}$=87.8; p<0.001, η^2=0.33), and the P/T/ΣLNAA ratio ($F_{(1,15)}$=101.8; p<.001, η^2=0.14), reflecting greater reductions for the P/T[−] beverage relative to control. Simple effects analyses demonstrated that consumption of the P/T[−] beverage reduced the serum concentration of phenylalanine by an average of 45.6% (95% CI: 42.2–48.9%; $t_{(15)}$=12.91, p<.001) and the serum concentration of tyrosine by an average of 47.9% (95% CI: 45.7–50.1%; $t_{(15)}$=13.26, p<.001). In contrast, consumption of the control beverage *increased* the serum concentration of phenylalanine by an average of 32.6% (95% CI: 25.4–39.8%; $t_{(15)}$=−4.79, p<.001) and the serum concentration of tyrosine by an average of 89.5% (95% CI: 75.2–1.04%; $t_{(15)}$=−6.41, p<.001) in the blood. Finally, consumption of the P/T[−] beverage decreased the P/T/ΣLNAA ratio by an average of 79.2% (95% CI: 76.7–81.7%; $t_{(15)}$=20.2, p<0.001), while the control beverage decreased the P/T/ΣLNAA ratio by an average of 27% (95% CI: 22.6–31.4%; $t_{(15)}$=5.57, p<0.001). These data demonstrate the efficacy of our P/T-depletion protocol, and based on the relationship between the peripheral P/T/ΣLNAA ratio and DA availability within the brain (Montgomery *et al.*, 2003), we infer successful reduction of central DA.

3.2 Effects of P/T depletion on blood pressure and mood state

P/T depletion has been reported to cause decreases in systolic and diastolic blood pressure (Moja *et al.*, 1996). However, we observed no significant effect on either systolic blood pressure (time × beverage interaction, $F_{(1,15)}$=0.47; p=0.50, η^2=0.01) or diastolic blood

pressure (time × beverage interaction, $F_{(1,15)}$=0.08; p=0.79, η^2=0). The amino acid beverage formulation in Moja and colleagues' study included only seven amino acids, while the formulation used here includes sixteen amino acids. Thus, differences in beverage formulations could account for the differing effects on blood pressure.

A recent meta-analysis found that P/T depletion does not alter mood in healthy controls (Ruhe *et al.*, 2007), which we confirmed here. Participants completed the POMS questionnaire before consuming the amino acid beverage and at session end. A time by beverage repeated measures ANOVA found no significant interacting effect on POMS scores ($F_{(1,15)}$=0.17; p=0.69, η^2=0). We also found no main effects of time or beverage (max. F=0.48, min. p=0.5).

3.3 Age-dependent effects of P/T depletion on learning from punishment

To quantify participants' ability to learn from punishment, we calculated incorrect responses to "bad" stimuli, so called "passive avoidance errors" (PAEs). A mixed measured ANOVA (beverage × task block), found no significant effect of P/T depletion on PAEs ($F_{(1,15)}$ = 0.68, p = 0.42; Fig. 1a). We did find a significant effect of task block ($F_{(8,120)}$=33.6; p<0.001, η^2=0.45), reflecting a decrease in PAEs across blocks, indicating successful learning to avoid punishment (Fig. 1b). We detected no significant beverage × block interaction ($F_{(8,120)}$=0.86; p=0.55, η^2=0.01; Fig. 1b), indicating that P/T depletion did not affect the rate at which participants learned from punishment.

A variety of evidence suggests age-dependent changes in DA signaling in emerging adults relative to "full" adults (Smith and Boettiger, 2012, Tunbridge *et al.*, 2007, Wahlstrom *et al.*, 2010). Therefore, we evaluated whether P/T depletion effects on PAE interacted with age. First, considering age as a continuous variable, we found that P/T depletion reduced the rate of PAEs with increasing age by 1.49 PAEs/year (95% CI, −0.71–−2.27, r=−0.74, p=0.001, Fig. 1c). In other words, P/T depletion tended to reduce the PAEs of older participants, while it tended to increase the PAE among emerging adult participants. When age was included as a covariate in our repeated measures ANOVA (beverage × block), we found a significant effect of beverage on PAEs ($F_{(1,14)}$ = 14.66, p = .002, η^2=0.09), as well as a significant beverage*age interaction ($F_{(1,14)}$=16.75, p=0.001, η^2=0.10). Examining PAEs in the control beverage session as a function of age, we found 1.22 additional PAEs with each additional year of age (95% CI, 0.24–2.21, r=0.58, p=0.019). In other words, on the control beverage, increasing age was associated with decreased ability to learn from punishment.

We next separated participants into emerging adult (22–25 years old) and adult (26–40 years old) groups to determine whether the response to dopamine depletion differed among emerging adults. We found a significant beverage by age group interaction (FS=18.35; p=0.001, η^2=0.07; Fig. 1d), which reflected the fact that P/T depletion reduced PAEs among the adults ($t_{(8)}$=3.2, p=0.007, Cohen's d=−0.88), while it increased PAEs among the emerging adults ($t_{(6)}$=−3.6, p=0.006, Cohen's d=−0.60). A comparison of the emerging adult and adult groups found no significant differences (aside from age) in their demographics, personal and familial substance use, or psychometric measures (Table 1).

3.4 Age-dependent effects of P/T depletion on reward learning

In the PAL task, in addition to learning from punishment, participants also learn from reward, which is quantified in terms of omission errors (OEs). Taking OEs as our dependent measure, a mixed measured ANOVA (beverage × task block) found no significant main effect of beverage ($F_{(1,15)}$= 0.82, p=0.38, η^2=0.03; Fig. 2a) or task block ($F_{(2.97, 56.07)}$=1.19, p=0.32, η^2=0.02), and no significant beverage by block interaction ($F_{(3.76,56.37)}$=1.26; p=0.30, η^2=0.02; Fig. 2b).

Given the age moderation of P/T depletion effects on PAEs, we examined whether the effect of P/T depletion on OE performance also varied with age. In considering age as a continuous variable, we found a small, non-significant decline in OEs with increasing age (0.30 errors/year; 95% CI: −1.45–0.84, r=−0.15, p=0.58; Fig. 2c). When we included age as a covariate in our beverage × block ANOVA, we found no significant main or interacting effects of age on OEs (max. F=1.07; min. p=0.38). Examining control session performance as a function of age, in contrast to the PAE data, we found no significant correlation between OEs and age (r=0.093, p=0.73). When participants were separated into emerging adult and adult groups (as for the PAE analysis in the previous section), we found a large and significant beverage by age group interaction effect on total OEs ($F_{(1,14)}$=5.57; p=0.033, η^2=0.26; Fig. 2d), which reflected a trend toward 10-fold greater OEs after P/T depletion among emerging adults ($t_{(6)}$=−2.45, p=0.075, Cohen's d=6.5). In contrast, adults showed a small, statistically insignificant decrease in OEs following P/T depletion ($t_{(8)}$=0.90, p=0.40, Cohen's d=−0.39).

3.5 Effects of P/T depletion on reaction times

Profound DA depletion should induce Parkinsonian motor deficits, which could confound detection of cognitive effects. Thus, we assessed the effect of P/T depletion on aspects of motor function (Table 2). We found no significant main effect of P/T depletion on overall task RT ($F_{(1,15)}$=0.05; p=0.83, η^2=0); this finding was not altered by including age as either a covariate ($F_{(1,14)}$=0.46; p=0.51, η^2=0.03) or a factor ($F_{(1,14)}$=0.01; p=0.94, η^2=0). We also observed no main or interacting effects of age on overall RT (max. F=1.39, min. p=0.26). We next investigated whether individual differences in P/T depletion effects on RT correlated with effects on PAL task performance. We found that P/T depletion effects on RT were positively correlated with effect on OEs (r=0.60, p=0.01). In other words, more slowing after P/T depletion was associated with more OEs. A similar trend was seen in the relationship between P/T depletion effects on PAEs and RT (r=0.43, p=0.10).

In addition to investigating overall RT, we also examined effects of P/T depletion on post-error slowing, a marker of response-monitoring (Dutilh *et al.*, 2012, Rabbitt, 1966). We found that P/T depletion had no significant effects on post-error response RT, post-correct response RT, or post-error slowing, either in the sample as a whole or within the adult or emerging adult groups (Table 2). Likewise, an age group by beverage ANOVA found no significant main or interacting effects on post-error slowing (max. F=1.19, min. p=0.30). After accounting for effects of age, we found no relationship between P/T depletion effects on post-error slowing and OEs (β= −0.33, t=−1.21, p=0.25); however, P/T depletion-induced change in post-error slowing negatively correlated with the change in PAEs (β= −0.38, t=

−2.44, p=0.031). Examining post-error and post-correct RTs separately, after accounting for effects of age, we observed a relationship between the effect of P/T depletion on OEs and on the post-correct response RT (Table 3). We also found a positive relationship between the effect of P/T depletion on PAEs and on the post-correct response RT (Table 3). Thus, the more that P/T depletion slowed RT after correct responses, the more P/T depletion increased errors. In contrast, we found no relationship between the effect of P/T depletion on post-error response RT and either OEs (β=0.06, t=0.2, p=0.85) or PAEs (β= −0.15, t=−0.81, p=0.44).

3.6 Relationship between P/T depletion effects on mood state on and PAL task performance

Based on our finding that P/T depletion appears to diminish reward-learning, possibly by dampening normal responses to positive feedback, we investigated whether P/T depletion effects on mood state could also predict learning performance. When the effect of P/T depletion on POMS scores was added as a regressor in a model predicting change in OEs in the whole sample, it did not predict significant variance (β= −0.08, t=−0.36, p=0.73) beyond that predicted by age and change in post-correct RT. We also found no significant relationship between the effect of P/T depletion on PAEs and on POMS scores (β=0.12, t=0.75, p=0.47). When considering the age groups separately, among adults, we again found no significant relationships between changes in mood and changes in errors (min. p=0.3). In contrast, within the emerging adult group, we found that change in POMS scores predicted substantial variance in P/T depletion effect on OEs (β= −0.78, t=−4.28, p=0.023), after accounting for age and change in post-correct RT change. Thus, among emerging adults, greater declines in mood following P/T depletion resulted in more OE following P/T depletion. No such relationship was observed between POMS scores and PAEs among emerging adults (β= −0.25, t=−0.53, p=0.63).

4. DISCUSSION

4.1 Dopamine depletion and sensitivity to punishment

A variety of evidence supports the idea that reducing striatal DA signaling improves learning from negative feedback, or punishment, whereas increases in striatal DA signaling may improve learning from positive feedback, or reward (Cools *et al.*, 2006, Cools *et al.*, 2009, Frank, 2005, Frank *et al.*, 2004, Moustafa *et al.*, 2008, Pessiglione *et al.*, 2006, Robinson *et al.*, 2010, Shohamy *et al.*, 2008). Given the decline in DA signaling following late adolescence (Wahlstrom et al., 2010), one would expect to find age-dependent improvement in learning from punishment, which is what we observed here: the ability to learn from negative feedback in the control session correlated with age: the older participants were, the better they learned from punishment. In addition, acute reduction in central DA via P/T depletion improved learning from punishment among adults. Here again we observed age-dependent effects, where the older participants were, the more DA depletion improved their ability to learn from punishment. Paradoxically, however, among emerging adults, acute DA depletion actually impaired learning from punishment. The degree to which learning from punishment was impaired by DA depletion was predicted by the degree to which DA depletion slowed RT after correct responses. Such trial-to-trial

changes in RT during feedback-based learning may reflect DA depletion effects in extrastriatal sites, such as the prefrontal cortex (Moustafa *et al.*, 2008). Why emerging adults would be more sensitive to DA depletion in extrastriatal sites remains unclear. One possibility is that baseline differences in frontal DA tone may differ between adults and emerging adults. While data from non-human primates indicate that DA enervation of the PFC peaks in adolescence (Lambe *et al.*, 2000, Rosenberg and Lewis, 1995), and DA receptor expression reaches adult levels in adolescence (Lidow and Rakic, 1992), expression of the catechol-o-methyltransferase (COMT) enzyme increases from emerging adulthood to adulthood (Tunbridge *et al.*, 2007). As COMT catabolism of DA regulates tonic DA levels in the PFC (Gogos *et al.*, 1998, Karoum *et al.*, 1994, Tunbridge *et al.*, 2004, Wu *et al.*, 2012), emerging adults may have elevated levels of tonic DA in the PFC relative to adults. To our knowledge, this hypothesis has not been tested in human subjects. Moreover, while P/T depletion decreases both tonic DA and DA release in the striatum of adults (Leyton *et al.*, 2004, Montgomery *et al.*, 2003), no data is available regarding its effect on either tonic or phasic DA in the PFC, and no data is available regarding P/T depletion effects in emerging adults.

4.2 Dopamine depletion and sensitivity to reward

It is unclear why P/T depletion causes an improvement in PAL performance in adults and a worsening in PAL performance in emerging adults. Among emerging adults, we found that those who experienced a more negative mood state following P/T-depletion were also more likely to fail to respond to rewarding stimuli following P/T-depletion. We observed no such relationship between P/T-depletion effects on mood and reward-sensitivity in adults. Therefore, it is possible that the effect of reducing DA in emerging adults is not purely cognitive and also involves an affective aspect. Given data showing developmental changes in DA signaling (Kaasinen *et al.*, 2002, Kaasinen *et al.*, 2000), our data suggest that age-related differences in DA signaling underlie developmental changes in reward-sensitivity (Jarcho *et al.*, 2012). Our data suggest that emerging adults are more sensitive to dysphoric effects of acute P/Tdepletion, which induces a reduction in DA signaling. As dopamine depletion has long been hypothesized to serve as the physiological basis of cocaine dependence (Dackis and Gold, 1985), this may have implications for substance abuse risk. Indeed, loss of phasic dopamine has recently been proposed as a marker of addiction (Caprioli *et al.*, 2014) on the basis of recent studies in rodents (Willuhn *et al.*, 2014). Notably, emerging adulthood is associated with both peak experimental drug use (Kandel and Logan, 1984) and the peak of substance use disorder onset (Kessler *et al.*, 2005). It is tempting to speculate that peculiarities of the emerging adult dopamine system render this age cohort more vulnerable to the dopamine depleting effects of drugs of abuse, which may in turn drive the compulsive use that can lead to addiction.

4.3 Motor effects of DA depletion

There was a relationship between the effect of the reduction in DA on PAL task performance and RT, where the individuals who performed poorly following DA-depletion also slowed their RT following DA-depletion. This RT effect appears to be specifically driven by the post-correct response RT, where individuals slowed down after making a correct response following DA-depletion. One possible explanation for this effect is that

reduced DA levels rendered participants more distracted by rewarding feedback, which in turn could slow their RT on the next trial. These motor effects provide a confirmation that central DA is being depleted by P/T-depletion.

4.4 Study limitations

We acknowledge some limitations of the present study. First, our sample size is rather modest, so although some of effect sizes were rather large, these findings bear replicating. Moreover, any negative findings here may result from lack of power. In addition, we acknowledge some limitations to the method we used to manipulate DA, acute P/T-depletion. Although studies in rats (McTavish, Cowen, & Sharp, 1999) and humans (Leyton et al., 2004) show that P/T-depletion reduces DA release in the striatum by 30% or more, lowering DA levels in the brainstem could theoretically reduce DRD2-mediated autoinhibition of DA neurons, thereby increasing DA release in other projection regions. However, human PET studies demonstrate that the degree to which P/T-depletion reduces DA receptor occupancy in the striatum predicts the level of executive function impairment (Mehta, Gumaste, Montgomery, McTavish, & Grasby, 2005); P/T-depletion effects on extrastriatal targets have not been adequately investigated. Beyond changes in DA within target regions, P/T-depletion reduces frontostriatal connectivity (Nagano-Saito et al., 2008). While both rodent and human studies suggest that P/T-depletion does not affect norepinephrine (NE) dependent processes (Leyton et al., 2004; McTavish, Callado, Cowen, & Sharp, 1999; McTavish et al., 2001; B. Sheehan et al., 1996), one study found that P/T depletion reduces levels of a NE metabolite (Palmour, Ervin, Baker, & Young, 1998). However, acute P/T-depletion does not affect NE-regulated melatonin levels, while it does alter DA-regulated prolactin levels (Harmer et al., 2001; B. Sheehan et al., 1996). Therefore, while P/T-depletion effects on NE cannot be completely ruled out, available evidence suggests that any such effects are relatively minimal. Another limitation of P/T-depletion is that it is subtler than the profound global forebrain DA depletions used in animals. Given the profound dependence of motor function on DA, the subtlety of the effects of P/T-depletion on cognition are advantageous in that it allows detection of behavioral effects without confounding motor impairment.

CONCLUSIONS

In a sample of healthy adults, the effects of acute reduction in DA signaling on positive- and negative-reinforcement based learning in the deterministic PAL task was modulated by age. Specifically, with DA depletion had increasingly beneficial effects on learning from punishment with increasing age. In this small sample, DA depletion impaired PAL performance in emerging adults (ages 22–25), but improved PAL performance in more mature adults (ages 26–40). Effects on learning from reward were qualitatively similar, but did not reach statistical significance; this negative finding may reflect a lack of statistical power to detect an effect. In addition, while we failed to detect a global slowing of RT's, those who responded more slowly after DA depletion also made more errors in the DA depletion condition. Moreover, when DA depletion slowed RT after correct responses, PAL task performance was degraded; this was specific to the positive feedback condition. Finally, among emerging adults, changes in mood following DA depletion negatively correlated with

learning from positive feedback. Together, these data indicate that both positive- and negative-feedback based learning are sensitive to central catecholamine levels, and that these effects of acute DA reduction vary with age.

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Highlights

- Dopamine depletion effects on passive avoidance learning are modulated by age.
- Dopamine depletion improves learning from punishment with increasing age.
- In emerging adults, depleting dopamine affects reward learning together with mood.

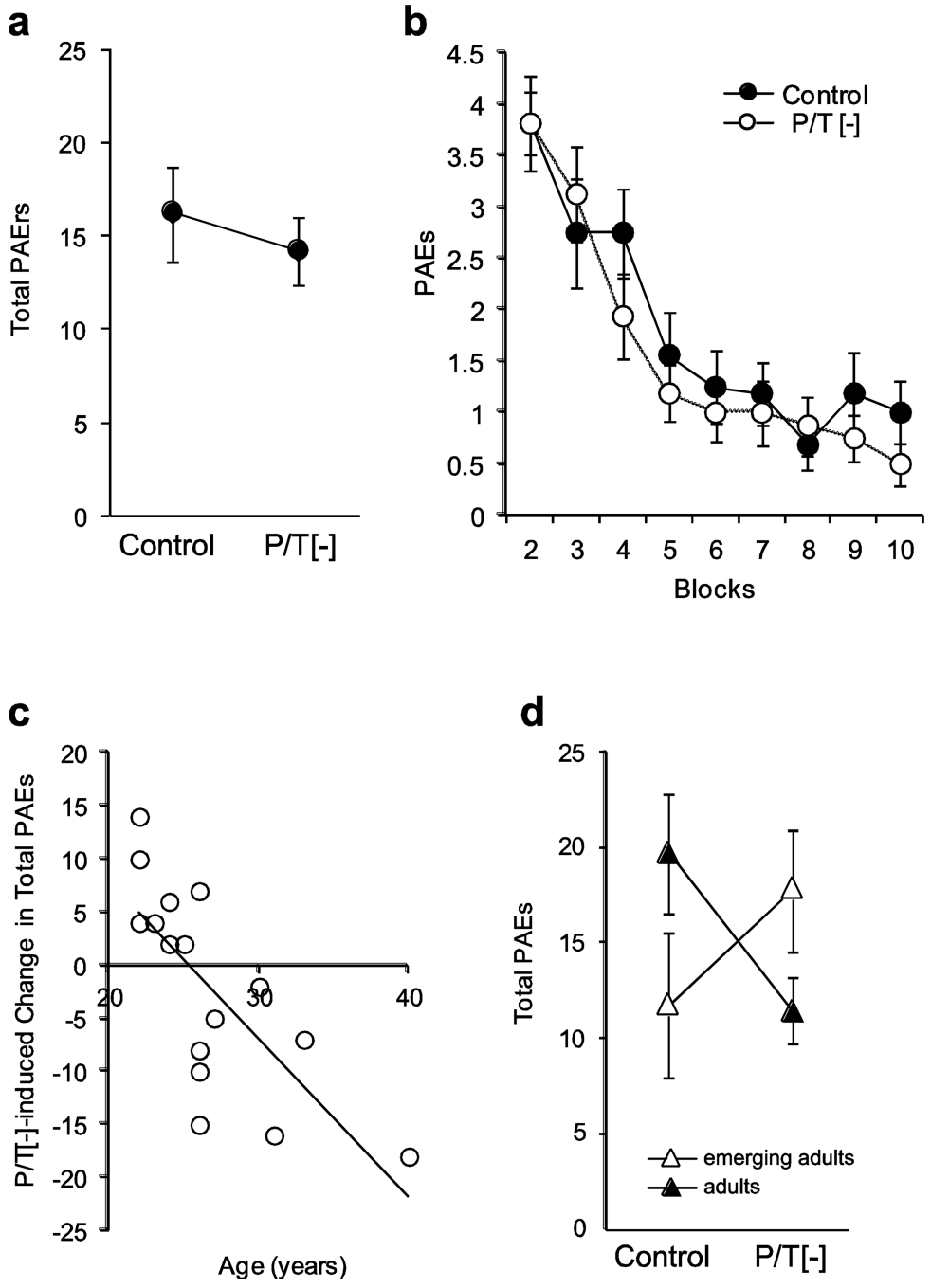

Figure 1.

Effect of P/T depletion on PAEs is age dependent. *a*) There is no effect of P/T depletion on total PAEs *b*) There is no effect of P/T depletion on PAEs when the data are separated by block. *c*) There is a significant correlation between the age of the participant and the effect of P/T depletion on PAEs (95% CI, −0.71– −2.27, r=−0.74, p=0.001). *d*) When participants are separated into emerging adult (22–25 years old) and adult (26–40 years old) groups, there is a significant beverage×group interaction ($F_{(1,14)}$=18.35; p=0.001, η^2=0.07), where the adult participants (n = 9) perform better on the P/T[−] beverage ($t_{(8)}$=3.2, p=0.007,

Cohen's d=−0.88), and the emerging adult participants (n = 7) perform worse ($t_{(6)}$=−3.6, p=0.006, Cohen's d=−0.60). PAEs, Passive Avoidance Errors; P/T[-], phenylalanine/tyrosine-depleted beverage.

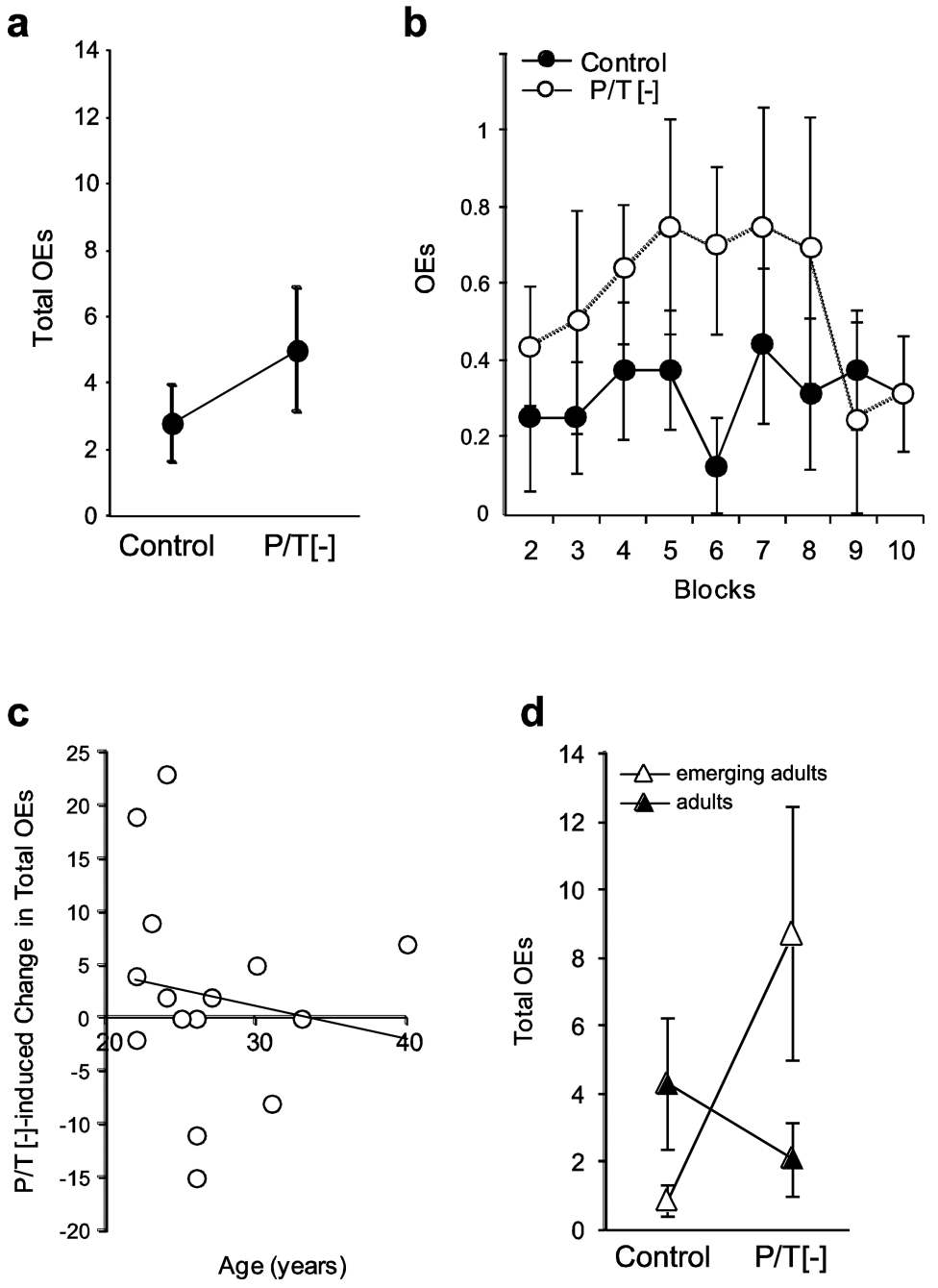

Figure 2.

Effect of P/T depletion on OEs is age dependent. *a*) There is no effect of P/T depletion on total OEs *b*) There is no significant beverage × block interaction on OEs. *c*) There is not a significant correlation between the age of the participant and the effect of P/T depletion on PAEs. *d*) When participants are separated into emerging adult (22–25 years old) and adult (26–40 years old) groups, there is a significant beverage × age group interaction

($F_{(1,14)}$=5.57; p=0.033, η^2=0.26). OEs, Omission Errors; P/T[−], phenylalanine/tyrosine-depleted beverage.

Table 1

A comparison of the emerging adult (22–25 years old) and adult groups (26–40 years old).

	Emerging Adults (n = 7)	Adults (n = 9)	$t_{(14)}$	p value
General				
Age	23.14 ± 0.46	29.44 ± 1.58	−3.83[a]	0.004
SES	52.25 ± 1.62	53.64 ± 2.46	*−0.44*	*ns*
Gender (% female)	28.6	33.3		*ns*[†]
Ethnicity (% non-white)	14.3	33.3		*ns*[†]
Personal and Familial Substance Use				
AUDIT	4.29 ± 0.87	5.56 ± 1.24	−0.79	*ns*
DUSI	0.12 ± 0.04	0.11 ± 0.05	0.22	*ns*
FTQ	0.23 ± 0.06	0.13 ± 0.06	1.13[b]	*ns*
Psychometric Measures				
BIS	59.00 ± 1.84	57.56 ± 1.59	0.60	*ns*
FTPI-Mean Extension	11.70 ± 3.31	8.78 ± 2.42	0.73	*ns*
FTP1-Max Extension	42.29 ± 9.46	35.44 ± 9.85	0.49	*ns*
MMPI	8.00 ± 1.23	6.78 ± 1.27	0.68	*ns*
BDI	3.86 ± 1.06	3.00 ± 0.82	0.65	*ns*
STAI-Trait Score	30.29 ± 1.39	34.78 ± 2.72	−1.35	*ns*
LOC	11.71 ± 0.68	10.11 ± 1.09	1.25[c]	*ns*

Table 2

The effect of P/T depletion on RT

	Control	P/T[−]		
Full Sample			$t_{(15)}$	*p* value
Overall Reaction Time (RT)	953.2 ± 32.9	945.8 ± 27.7	0.22	0.83
Post Error Response RT	995.4 ± 52.0	943.8 ± 45.5	0.75[a]	0.46
Post Correct Response RT	951.9 ± 32.9	965.9 ± 32.1	−0.31	0.76
Post Error Slowing	30.7 ± 46.8	−31.3 ± 51.9	0.98	0.35
Emerging Adults			$t_{(12)}$	*p* value
Overall RT	917.2 ± 34.3	954.3 ± 36.6	−0.74	0.47
Post Error Response RT	1004.9 ± 90.2	948.9 ± 74.1	0.48	0.64
Post Correct Response RT	911.4 ± 34.1	990.7 ± 44.3	−1.42	0.18
Post Error Slowing	93 ± 85.6	−41.9 ± 109.3	1.24	0.26
Adults			$t_{(16)}$	*p* value
Overall RT	981.2 ± 51.9	939.1 ± 42.0	0.63	0.54
Post Error Response RT	987.0 ± 63.3	939.3 ± 60.5	0.55[b]	0.59
Post Correct Response RT	983.3 ± 51.4	946.6 ± 46.8	0.53	0.60
Post Error Slowing	−24.3 ± 42.3	−22.0 ± 69.9	−0.03	0.97

Values are reported as mean ± standard deviation. Reported *p*-values reflect the results of paired two-tailed comparisons for the effect of the beverage. Exact *p*-values reported unless $p < 0.001$.

[a] $df=28$,

[b] $df=14$.

Table 3

Factors Predicting the Effect of P/T-depletion on PAL Task Performance

	B	SE B	β	*p*
Omission Errors				
Full sample model				
Constant	2.10	10.8		0.849
Age	−0.001	0.398	−0.001	0.997
Post-correct RT change	**0.046**	**0.014**	**0.705**	**0.006**
POMS change	−0.085	0.237	−0.075	0.726
Adults				
Constant	−15.7	9.95		0.176
Age	0.522	0.332	.399	0.176
Post-correct RT change	0.025	0.011	.568	0.067
POMS change	0.226	0.197	.293	0.302
Emerging Adults				
Constant	−27.1	32.4		0.464
Age	1.54	1.43	.193	0.360
Post-correct RT change	0.040	0.015	.454	0.075
POMS change	**−1.02**	**0.239**	**−.780**	**0.023**
Passive Avoidance Errors				
Full sample model				
Constant	31.9	8.60		0.003
Age	**−1.29**	**0.316**	**−0.640**	**0.002**
Post-correct RT change	**0.029**	**0.011**	**0.403**	**0.026**
POMS change	0.141	0.188	0.115	0.470
Adults				
Constant	20.1	13.9		0.208
Age	−0.915	0.464	−0.556	0.106
Post-correct RT change	0.035	0.015	0.624	0.070
POMS change	0.130	0.275	0.134	0.656
Emerging Adults				
Constant	54.8	38.3		0.248
Age	−2.07	1.69	−0.562	0.307
Post-correct RT change	−0.005	0.018	−0.111	0.815
POMS change	−0.150	0.283	−0.248	0.632

Results from multiple linear regression analysis of predictors of the P/T-depletion effect on PAL task performance.

B: beta value; SE B: beta value standard error; β: standardized beta.