Amphetamine alters an EEG marker of reward processing in humans and mice

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Abstract

The bench-to-bedside development of pro-cognitive therapeutics for psychiatric disorders has been mired by translational failures. This is in part due to the absence of pharmacologically-sensitive cognitive biomarkers common to humans and rodents. Here, we describe a cross-species translational marker of reward processing that is sensitive to the dopamine agonist, d-amphetamine. Motivated by human electroencephalographic (EEG) findings, we recently reported that frontal midline delta-band power is also an electrophysiological biomarker of reward surprise in mice. In the current series of experiments, we determined the impact of parametric doses of d-amphetamine on this reward-related EEG response from humans (n=23) and mice (n=28) performing a probabilistic learning task. In humans, d-amphetamine (placebo, 10 mg, 20 mg) boosted the Reward Positivity event-related potential (ERP) component as well as the spectral delta-band representations of this signal. In mice, d-amphetamine (placebo, 0.1 mg/kg, 0.3 mg/kg, 1.0 mg/kg) boosted both reward and punishment ERP features, yet there was no modulation of spectral activities. In sum, the present results confirm the role of dopamine in the generation of the Reward Positivity in humans, and paves the way towards a pharmacologically valid biomarker of reward sensitivity across species.

Keywords: d-amphetamine, Reward Positivity, reinforcement learning, EEG, mouse, human

63 **Introduction**

64 Animal models of cognitive processes have been beset by translational difficulties. We
65 recently addressed this problem by identifying common electrophysiological biomarkers of
66 isolated cognitive processes in both humans and mice (Cavanagh et al. 2021). One of the most
67 promising outcomes identified in that project was a common delta-band spectral reflection of the
68 *Reward Positivity* (RewP), an event-related potential (ERP) component that has been well-
69 described in human participants (Holroyd et al. 2008; Proudfit 2015). While the discovery of cross-
70 species biomarkers is an important step, their translational utility requires a demonstration of
71 consistent pharmacological predictive validity (i.e. similar drug effects across species). Here, we
72 tested whether this cortical reward signal had similar sensitivity to d-amphetamine in mice and
73 humans.

74 The RewP is a positive deflection in the ERP that is most commonly quantified over fronto-
75 central sites around 200-400ms after reward presentation (Holroyd et al. 2011; Proudfit 2015;
76 Heydari and Holroyd 2016). Both the RewP and its delta-band spectral reflection scale with the
77 degree of positive reward prediction error, whereby "better than expected" outcomes evoke
78 increasingly larger RewP amplitudes (Baker and Holroyd 2011; Cavanagh 2015; Holroyd and
79 Umemoto 2016). These two criteria are notable: they indicate that this reward-related signal is
80 specific and sensitive to positive reward prediction error, fulfilling the stringent criteria of being an
81 invariant neural marker of this computational process (Cacioppo and Tassinary 1990).

82 The RewP is diminished in disorders like major depression (Bress et al. 2013; Webb et al.
83 2016) and Parkinson's disease (Brown et al. 2020b), suggesting it might be a trans-diagnostic
84 biomarker of reward responsiveness, learning, and valuation. The emerging mechanistic
85 understanding of this signal increases its appeal: the reward prediction error computation
86 reflected by the RewP mirrors the dopamine-driven learning process underlying reinforcement
87 learning (Sutton and Barto 1998). The cortically-generated RewP has been theorized to be
88 modulated by phasic midbrain dopamine (Holroyd et al. 2008), however empirical tests of this

89 have been sparse and beset by methodological complications (see discussion), with little
90 evidence for pharmacological predictive validity across species. It remains unknown if these
91 cortical and midbrain systems influence each other in a causal manner, if they are both influenced
92 by a third variable, or if they reflect parallel processes. Despite these uncertainties, the current
93 understanding of the RewP suggests that it should be affected by manipulations of dopaminergic
94 activity. The current study aimed to provide an initial test of this intuitive hypothesis, as well as
95 facilitate the first test of common underlying mechanisms in the homologous mouse cortical
96 signal.
97
98 **Materials and Methods**
99 *Human Participants*
100 Healthy women and men between the ages 18-35 years were recruited from the
101 community via public advertisements and compensated monetarily for study participation. This
102 study was conducted at the UCSD Medical Center and was approved by the UCSD Human
103 Subject Institutional Review Board. Participants first completed a phone screen to assess current
104 and past medical and psychiatric history, medication and recreational drug use and family history
105 of psychosis. During the subsequent in-person screening visit, participants signed the consent
106 form and completed assessments of physical and mental health, including a structured clinical
107 interview, self-report questionnaires about caffeine intake and handedness, a hearing test,
108 physical examination, an electrocardiogram, urine toxicology screen, urine pregnancy test for
109 females, the MATRICS Comprehensive Cognitive Battery (MCCB) and a Wide Range
110 Achievement Test (WRAT) for IQ assessment. See Supplemental Table 1 for these clinical
111 demographics. A total of 12 male and 11 female participants completed all three sessions and
112 are included here.
113 A double blind, randomized, placebo-controlled, counterbalanced, within-subject design
114 was utilized. Participants received either placebo or one of two active doses of d-amphetamine

115 (10 or 20 mg) orally on each of the three test days which were separated by one week. On all
116 assessment days, participants arrived at 8:30 am after overnight fasting, completed a urine
117 toxicology screen and a urine pregnancy test in females, and ate a standardized breakfast. Vital
118 signs and subjective symptom rating scale scores were obtained at specific intervals pre- and
119 post- administration. Starting 120 minutes post-administration, subjects completed cognitive tests
120 and simultaneous EEG recording. See Supplemental Table 2 for experimental session
121 information.
122
123 *Probabilistic Learning Task (PLT)*
124 This version of the PLT was identical to our previous investigation (Cavanagh et al. 2021),
125 see Figure 1A. Participants were presented a stimulus pair (e.g., bicycle/phone, chair/clip,
126 plug/flashlight) on a computer monitor and instructed to select the "target" stimulus using a digital
127 4-switch USB arcade-style joystick. Participants were given feedback after each trial about
128 whether their response was "correct" or "incorrect". Reward probabilities for the target / non-
129 target stimulus were set within a block of 60 trials (80/20, 70/30, 60/40, 50/50), but stimuli differed
130 between trial blocks (first block was bicycle/phone at 80/20, then the next block was chair/clip at
131 60/40, etc.). Overall performance was calculated as the total number of correct target selections
132 in each block of 60 trials.

133 *Human Electrophysiological Recording and Pre-Processing*
134 Continuous electrophysiological (EEG) data were recorded in DC mode from 64 scalp
135 leads using a BioSemi Active Two system. Four electrooculograms (EOG) recorded at the
136 superior and inferior orbit of the left eye and outer canthi of each eye, and one nose and two
137 mastoid electrodes were also collected. All data were collected using a 1048 Hz sampling rate
138 utilizing a first-order anti-aliasing filter. Custom Matlab scripts and EEGLab (Delorme and Makeig
139 2004) functions were used for all data processing. Data were first down sampled to 500 Hz,

140 epoched around the imperative cues (-2000 to +5000 ms) and then average referenced. Bad
141 channels and bad epochs were identified and were subsequently interpolated and rejected
142 respectively, then blinks were removed following independent component analysis.
143
144 *Animal Subjects*
145 Female and male C57BL/6J mice were obtained from The Jackson Laboratory (Bar
146 Harbor, ME), housed in same sex groupings of 2 per cage in a temperature- and humidity-
147 controlled vivarium under a reverse 12 h light/dark cycle (lights off 0800 h) and tested during the
148 dark phase. All experimental procedures were performed in accordance with the National
149 Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the
150 University of New Mexico Health Sciences Center Institutional Animal Care and Use Committee.
151 A total of 15 male and 13 female mice were used. See Supplemental Methods for information on
152 touchscreen pre-training and surgery.
153 A randomized, placebo-controlled, counterbalanced, within-subject design was utilized.
154 Mice received either placebo or one of three active doses of d-amphetamine (0.1 mg/kg, 0.3
155 mg/kg, 1.0 mg/kg) on each of the four test days which were separated by a 72 hours washout
156 period. Starting 15 minutes post-administration, subjects completed the PLT with simultaneous
157 EEG recording. Notably, since 1.0 mg/kg leads to significantly increased hyperactivity and
158 significantly decreased task focus, it was investigated as an upper limit of dosing.
159
160 *Mouse PLT*
161 This version of the PLT was also identical to our previous investigation (Cavanagh et al.
162 2021), see Figure 1A. Each session, mice were presented with three pairs of unique stimuli
163 (fan/marble, spider/fan, honey/cave) in three separate 20 trial blocks, for a total for 60 trials per
164 session. For the first block, the target stimulus was rewarded 90% of the time and the non-target
165 stimulus was rewarded 10% of the time. The next blocks included 80/20 and then 70/30

reinforcement rates. The mice were given 120 minutes to complete the task. Rewards consisted of the immediate delivery of an auditory tone for 1 second signaling the availability of liquid reward (30μl strawberry Nesquik, Nestle). Punishments consisted of immediate illumination of the house light for 10 seconds before the next trial could be initiated.

Human and Mouse EEG Processing

For the sake of descriptive simplicity, both the scalp-recorded signal in humans as well as the dura-recorded signal in mice are referred to as 'EEG'. Time-Frequency measures were computed by multiplying the fast Fourier transformed (FFT) power spectrum of single trial EEG data with the FFT power spectrum of a set of complex Morlet wavelets defined as a Gaussian-windowed complex sine wave: $e^{i2\pi tf}e^{-t^2/(2x\sigma^2)}$, where t is time, f is frequency (which increased from 1-50Hz in 50 logarithmically spaced steps) and the width (or 'cycles') of each frequency band were set to increase from 3/(2πf) to 10/(2πf) as frequency increased. Then, the time series was recovered by computing the inverse FFT. The end result of this process is identical to time-domain signal convolution, and it resulted in estimates of instantaneous power taken from the magnitude of the analytic signal. Each epoch was then cut in length from -500 to +1000 ms peri-feedback.

Averaged power was normalized by conversion to a decibel (dB) scale (10*log10[power(t)/power(baseline)]), allowing a direct comparison of effects across frequency bands. The baseline consisted of averaged power -300 to -200 ms before all imperative cues. A 100 ms duration is often used as an effective baseline in spectral decomposition since pixel-wise time-frequency data points have already been resolved over smoothed temporal and frequency dimensions with the wavelets. For ERPs, a baseline correction of -300 to -200 ms was applied and data were 20 Hz low-pass filtered prior to averaging. Feedback-locked analysis was conducted at electrode FCz in humans and the frontal lead in rodents.

Statistical Analysis

Analytic methods are either identical to our previous report (Cavanagh et al. 2021), or they are new analyses. Analysis of the same time-frequency region of interest (tf-ROI) was proposed to be a major constraint within our 'learn-confirm' strategy between experimental phases (Cavanagh et al. 2021). ERPs were not reported in our prior 'learn phase' investigation; we now show the relevant mouse ERPs from that previous investigation in Supplemental Figure 1. We also report additional analyses that were facilitated by this novel 'confirm'-stage multi-session, within subject design. As in our previous report, hypotheses were specific to the reward-related EEG; however, punishment-related EEG signals are again reported here for consistency. The number of epochs for each condition are shown in Supplemental Figure 2.

Species were analyzed with separate mixed effects models. Each mouse was treated as a random effect, similar to humans. Drug condition and sex were treated as fixed effects, although there were no *a priori* hypotheses for sex effects. Given the established effects of dopamine agonism on frontal cortical activities, either linear or quadratic effects could be expected (depending on dose and individual differences). Fitting with the demands of parsimony and the inferential affordance allowed by the number of drug conditions, this resulted in linear contrasts for humans (placebo, 10 mg, 20 mg) but quadratic contrasts for mice (placebo, 0.1 mg/kg, 0.3 mg/kg, 1.0 mg/kg).

Scalar values of spectral power were derived from the same spatial, temporal, and frequency windows as in our foundational paper. For these expectation-based contrasts, comparisons were split based on the probabilistic aspect of the reward feedback, creating high probability (i.e. target response followed by reward) vs. low probability (i.e. non-target response followed by reward) contrasts. We adhered to our previously-defined tf-ROIs and we again omitted data from the 50/50 condition in humans (human reward: 1.3 Hz to 2 Hz, 250 to 550 ms; human punishment: 4 to 6.5 Hz, 450 to 750 ms; mouse reward: 1 to 4 Hz, 250 to 550 ms; mouse

punishment: 4.5 to 7.5 Hz, 500 to 800 ms). However, this study design offered an additional chance for inference.

Unlike our previous study, this multi-session design facilitated a comparison of similar feedback processes across levels of the fixed effect (drug) without the requirement of deriving a performance-based contrast to serve as a fixed contrast (expectation). This design enabled a comparison of the grand average feedback-related response outside the constraints of behavioral performance. This is beneficial, given the poor performance of mice on the task (Fig 1B). Critically, human feedback-locked EEG activities are most often assessed in un-learnable tasks with 50/50 reward probabilities (e.g. the two-door task: Foti and Hajcak 2009; Angus et al. 2015; Proudfit 2015; Mulligan and Hajcak 2017), highlighting how learning is not a necessary requirement for eliciting these cortical signatures of feedback receipt.

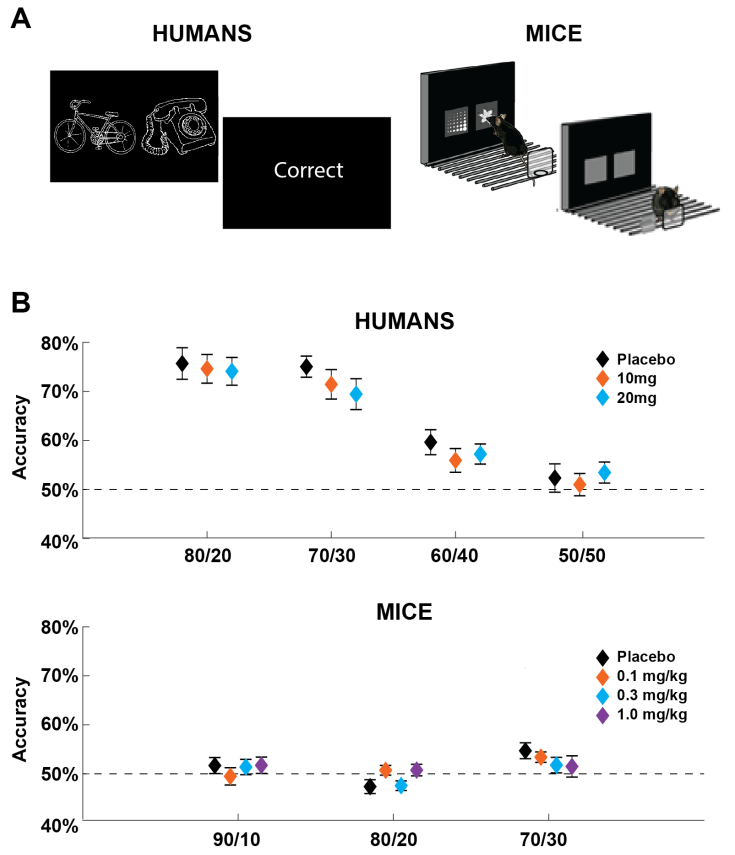

Figure 1. Task and performance. A) The Probabilistic Learning Task required the subject to select the stimulus that probabilistically led to reward most often. In humans and mice, each trial required a choice between two stimulus icons. B) Performance, split by block and drug. Humans displayed approximate matching behavior with no difference in performance due to amphetamine dose. In the 50/50 condition there was no chance to learn a 'better' stimulus and performance was at chance. Mice struggled to reliably discriminate between each pair of stimuli, but they did perform better than chance in aggregate.

Due to this affordance, here we investigated grand average ERPs for the first time. ERP components were *de novo* defined based on the grand average over all drug conditions (human reward:

242 250 to 400 ms, human punishment: 250 to 500 ms. mouse feedback: 400 to 600 ms), see Figure
243 2. In humans the grand average time-frequency activity peaked earlier than the expectation-
244 defined contrasts, so we defined new time ranges based on the grand average over all drug
245 conditions. For reward, this earlier window was from 100 to 400 ms and for punishment this
246 earlier window was 250 to 550 ms. There was no apparent simple grand average peak for
247 feedback in mice, so the previously defined tf-ROIs were used in all analyses.
248
249 **Results**
250 *Performance*
251 The full statistical results for performance data are reported in Supplemental Table 3. In
252 *humans*, there was a main effect of block (easier pairs were more accurate, see Figure 1B) and
253 a drug*sex interaction, which revealed a tendency for females to perform slightly worse under
254 higher amounts of d-amphetamine whereas males were unaffected (see Supplemental Fig 3). In
255 *mice*, there was no effect of block nor any effect with drug. This indicates that mice had poor
256 performance discriminating the probabilities between difficulty conditions, although they managed
257 to perform above chance when all drug and block conditions were collapsed (one sample t-test
258 vs. chance: t(27)=2.15, p=.04), see Figure 1B.
259
260 *ERPs*
261 All statistical results for ERP data are reported in Table 1. In *humans*, there was a main
262 effect of drug with a linear trend for increased RewP amplitude with increasing dose of d-
263 amphetamine. This effect was not present in the corresponding punishment condition, which
264 showed a trend towards a quadratic effect (although the quadratic contrast was not significant
265 either). In *mice*, there was a quadratic effect of drug on reward and punishment ERP amplitudes.
266 Since 1.0 mg/kg of d-amphetamine was at ceiling-level tolerance and is thus not ideally suitable
267 for comparison to humans, we examined if the same linear effect observed in humans was present

in the first three drug conditions alone. Similar to the pattern observed in humans, there was a linear effect of drug for the first three conditions for both reward and punishment ERP features.

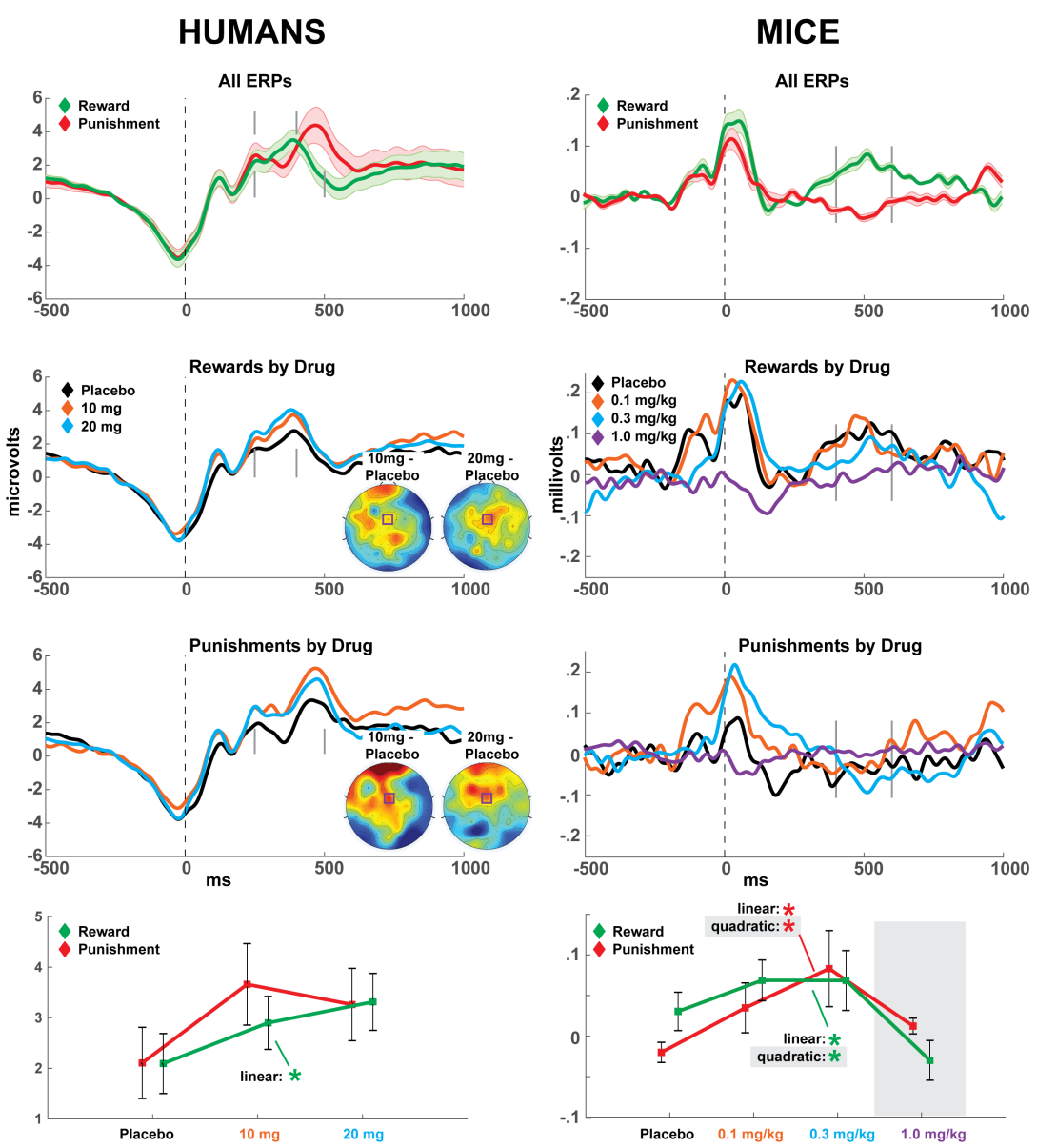

Figure 2. Feedback-locked ERPs from humans and mice. Grand average plots show feedback-locked ERP activity averaged over all drug conditions. Time windows for quantification are show with grey bars. Below, each feedback condition ERP is shown, split by drug dose. The same time window quantifications are shown with grey bars. Human ERPs topographical plots demonstrate drug condition differences; the purple square identifies the FCz electrode that was *a priori* chosen for analysis. Line plots (mean +/- SEM) detail the drug effects on each ERP component. In humans, only the RewP component was significantly affected by d-amphetamine. Mouse ERP features were characterized by quadratic effects with increasing d-amphetamine dose, however once the ceiling-level condition of 1.0 mg/kg was removed a similar linear trend as humans was also statistically identified. *p<.05 **p<.01.

		Main: Drug	Main: Sex	Drug*Sex	Drug*Drug	3-Way	Drug – no 1.0 mg
Human: Reward	ERP	**F=4.53 df=19.67 p=.046**	F=1.91 df=34.12 p=.18	F=.93 df=19.67 p=.35			
	tf-ROI Grand Average	**F=6.83 df=22.12 p=.02**	**F=10.42 df=20.99 p=.004**	F=1.92 df=22.12 p=.18			
	tf-ROI Expectation Contrast	**F=4.73 df=28.21 p=.04**	**F=8.73 df=34.33 p=.006**	**F=4.55 df=28.21 p=.04**			
Human: Punishment	ERP	F=3.04 df=18.69 p=.10	F=.39 df=29.80 p=.54	F=.49 df=18.69 p=.49			
	tf-ROI Grand Average	**F=8.30 df=23.36 p=.008**	F=.60 df=30.26 p=.45	F=2.06 df=23.36 p=.16			
	tf-ROI Expectation Contrast	F=2.84 df=32.68 p=.10	F=2.22 df=42.48 p=.14	F=1.63 df=32.68 p=.21			
Mouse: Reward	ERP	F=0.20 df=55.78 p=.66	F=2.19 df=38.61 p=.15	F=.09 df=55.78 p=.76	**F=5.62 df=36.28 p=.02**	F=0.84 df=36.28 p=.37	**F=5.58 df=22.75 p=.03**
	tf-ROI Grand Average	F=0.73 df=45.70 p=.40	F=1.17 df=60.45 p=.28	F=2.88 df=45.70 p=.10	F=1.14 df=72.28 p=.29	F=0.14 df=72.28 p=.71	F=0.97 df=25.66 p=.33
	tf-ROI Expectation Contrast	F=0.57 df=75.05 p=.46	F=0.36 df=60.93 p=.55	F=0.34 df=75.05 p=.56	F=1.73 df=102.08 p=.19	F=0.80 df=102.08 p=.37	F=.06 df=53.92 p=.81
Mouse: Punishment	ERP	F=3.81 df=51.22 p=.06	F=.33 df=48.16 p=.57	F=.47 df=51.22 p=.50	**F=4.19 df=57.23 p=.045**	F=0.76 df=57.23 p=.39	**F=6.14 df=46.19 p=.02**
	tf-ROI Grand Average	F=0.01 df=76.93 p=.95	F=1.55 df=52.77 p=.21	F=0.001 df=76.93 p=.97	F=0.00 df=65.48 p=.99	F=0.28 df=65.48 p=.60	F=0.04 df=38.24 p=.84
	tf-ROI Expectation Contrast	F=0.95 df=64.28 p=.33	F=0.05 df=59.78 p=.82	F=0.05 df=64.28 p=.82	F=1.15 df=70.07 p=.29	F=1.00 df=70.07 p=.32	F=4.03 df=37.19 p=.052

Table 1. Mixed linear model outcomes for ERP amplitudes, grand average tf-ROI power, and expectation-contrasted (low probability vs. high probability feedback) tf-ROI power. Quadratic effects were not tested in humans. An additional column details the main linear effect for drug dose in mice when omitting the 1.0 mg/kg (ceiling) condition in mice.

TF-ROI: Grand Average

All statistical results for grand average tf-ROIs are reported in Table 1. In *humans*, there were main effects of drug with linear trends for increased reward delta power and punishment

274 theta power with increasing dose of d-amphetamine, see Figure 3. For reward, there was also a
275 main effect of sex where males had higher reward delta power. In *mice*, there were no significant
276 outcomes for either reward or punishment.
277
278 *TF-ROI: Expectation*
279 All statistical results for tf-ROI expectation contrasts (low probability minus high
280 probability) are reported in Table 1. In *humans*, there were main effects of drug with a linear
281 trend for increased reward delta power with increasing dose of d-amphetamine. This effect was
282 not present in the corresponding punishment condition. There was also a main effect of sex
283 (higher power in males) and an interaction between drug and sex, where this expectation
284 difference declined slightly in males and increased in females with increasing dose of d-
285 amphetamine. In *mice*, there were no effects of d-amphetamine on tf-ROI expectation contrasts;
286 however this is likely due to the poor performance learning the behavioral discriminations (Figure
287 1B). In sum, only humans displayed an effect of d-amphetamine on reward-related tf-ROI power.
288
289 *Small-scale replication of mouse ERP findings*
290 We further aimed to replicate these appealing mouse ERP findings in a separate small-
291 scale cohort with easier learning discriminations (a single pair of stimuli: target 80% correct vs.
292 non-target 20% correct). Nine mice were run on placebo or 0.3 mg/kg of d-amphetamine across
293 multiple sessions. All mice learned to perform around 80% accuracy with no difference between
294 drug conditions. Supplemental Figure S4 shows that this drug-related enhancement of ERP
295 amplitude could be replicated, although it did not achieve statistical significance ($t(8)=1.64$,
296 $p=.14$, $d=.41$). However, the p-value is a poor metric for assessing replicability; effect sizes and
297 confidence intervals are more useful for assessing the utility of an experimental outcome
298 (Halsey et al. 2015; Colquhoun 2017). The effect size from this small-scale replication cohort
299 is larger than the one for cohort described in the main test (i.e. Figure 2): a paired t-test between

placebo and 0.3 mg/kg d-amphetamine on reward ERP amplitudes was t(27)=2.02, p=.05, *d*=.23.

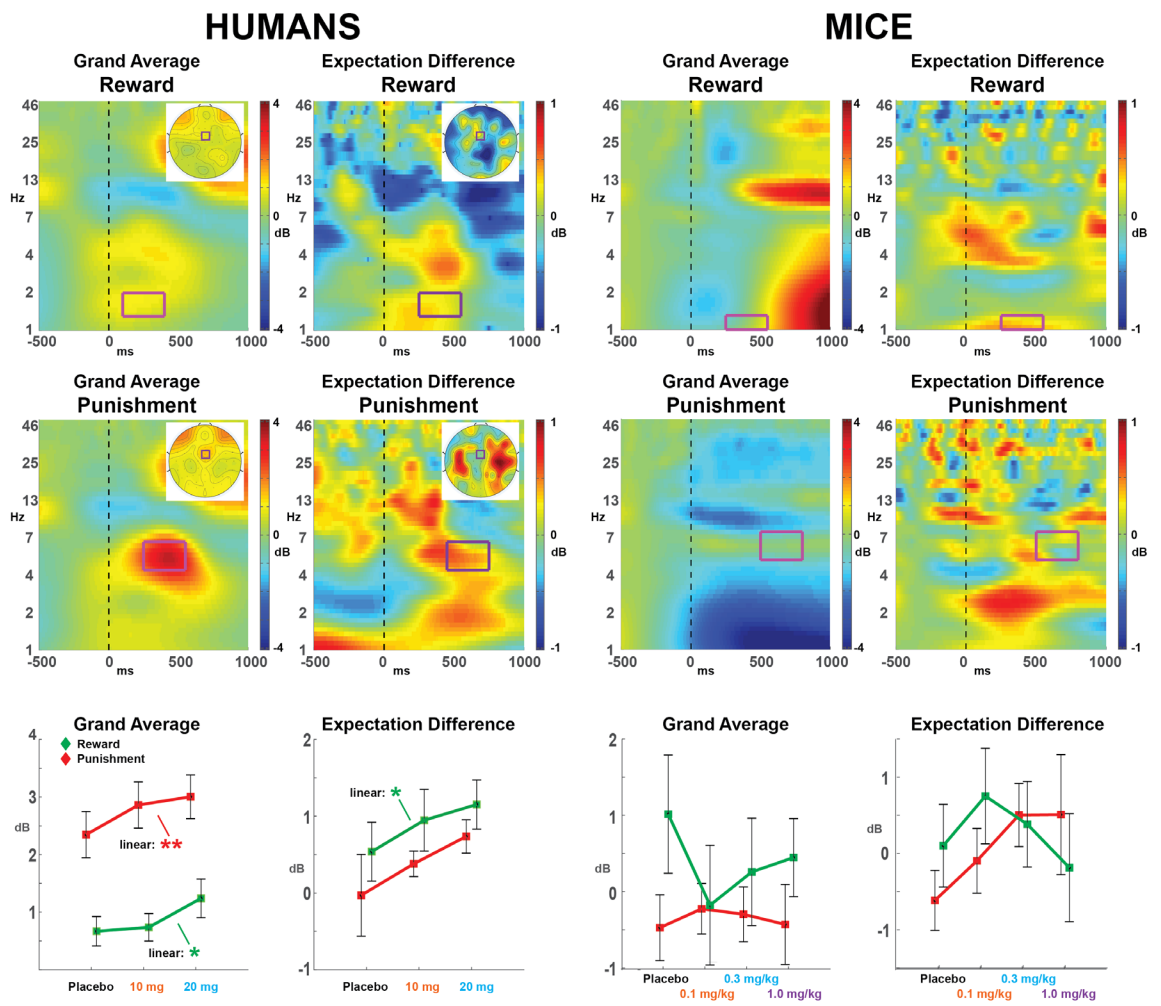

Figure 3. Time-frequency grand averages and expectation-related contrasts. Time-frequency plots of grand average and expectation difference (feedback from low vs. high probability conditions) at FCz in humans or the anterior lead in mice. The magenta box shows the tf-ROI. Human figures: grand average topographic plots: +/- 5 dB, expectation difference topographic plots: +/- 0.5 dB. Line plots (mean +/- SEM) detail the drug effects on each tf-ROI. *p<.05 **p<.01.

Discussion

This report validated our previous finding of a common electrophysiological marker of cortical reward processing in mice and humans as seen here during placebo (Cavanagh et al. 2021). The current work went further however, by demonstrating similar sensitivity of both species to increasing doses of d-amphetamine, demonstrating pharmacological predictive validity for this biomarker of reward sensitivity. While our previous cortical marker was based on the expectation-modulated spectral power, this study only found common cross-species amphetamine effects in the grand average ERP component. Unfortunately, the specific influence of d-amphetamine on learning-related spectral power in mice could not be definitively assessed due to poor learning. Yet, these findings already suggest that the influence of d-amphetamine is intrinsic to the generation of the RewP in both humans and mice.

As noted earlier, the RewP is sensitive to learning-related prediction errors, yet it is elicited by any rewarding feedback even in un-learnable environments (Foti and Hajcak 2009; Angus et al. 2015; Proudfit 2015; Mulligan and Hajcak 2017). This tendency appears to be preserved in the mice, who performed above chance but not at a level indicative of active successful learning. Our second small-scale replication cohort with good performing mice suggests that the enhancing effect of d-amphetamine on the RewP is reliable. However, two major issues remain to be addressed: 1) the specificity of d-amphetamine effects on reward vs. punishment conditions, and 2) the effect of d-amphetamine on reward signal generation vs. learning-related enhancements of this reward signal.

Specificity vs. Generality of d-Amphetamine Effects on Reward

In humans, there were general facilitating effects of d-amphetamine, although the effects were strongest for the reward-related conditions. The reward-related ERP, grand average tf-ROI, and expectation tf-ROI were all significantly affected by d-amphetamine in humans, whereas punishment-related effects were smaller and had some different overall trends than the RewP.

330 However, it is important to note that smaller effect sizes are not indicative of specific functional
331 dissociation. In mice, there were also general facilitating effects of d-amphetamine on reward
332 ERP amplitudes. Why did d-amphetamine affect spectral power in humans but not mice? One
333 reason might be the much lower peak frequency of the mouse delta band phenomenon, which
334 makes effective time-frequency quantification more difficult, particularly in the absence of a
335 learning-related contrast (see section below). In sum, the cumulative cross-species similarities
336 demonstrate that d-amphetamine boosts reward-related EEG signals in humans as well as mice,
337 although the specificity of this effect to rewards could not be definitively determined.

338 Previous evidence of dopaminergic sensitivity of these cortical signals in humans has also
339 been mixed, likely due to methodological reasons. Some studies have examined pharmacological
340 effects on the punishment-related EEG signal or the difference between reward and punishment
341 conditions, and have generally failed to find effects of the dopamine D2 receptor antagonists
342 haloperidol (Forster et al. 2017) or sulpiride (Mueller et al. 2014a; Lueckel et al. 2018) unless
343 moderated by genotype (Mueller et al. 2014b). However, conceptual issues about different
344 generative systems underlying reward and punishment EEG signals casts doubt on the suitability
345 of this common "difference wave" contrast (Meyer et al. 2017; Brown and Cavanagh 2020). One
346 experiment revealed a reduction in the RewP in response to the dopamine D2/3 receptor agonist
347 pramipexole (Santesso et al. 2009), yet there are no other studies of parametric or enhancing
348 effects of dopaminergic agents specific to the RewP in humans. A recent report observed that
349 although the RewP is specifically diminished in Parkinson's disease, L-dopa administration did
350 not alter this signal (Brown et al. 2020a). This surprising lack of acute dopaminergic influence
351 might be due to combined issues of cortical degeneration in Parkinson's and the differential
352 influence of L-dopa on striatal vs. cortical dopamine tone (Cools 2006). The findings from this

353 report extend the specificity of this extant literature in humans, demonstrating a clear influence of
354 cortical dopaminergic agonism on the RewP and associated EEG signatures of reward receipt.
355
356 *Reward Signal Generation vs. Learning-Related Enhancements*
357 Our previous investigation of cortical feedback signals was limited by the need to create
358 a well-controlled analytic contrast within each species (i.e. low vs. high probability outcomes
359 corresponding to high vs. low reinforcement prediction error), without interference from different
360 sensory or imperative stimuli (Cavanagh et al. 2021). Since rewarded actions for mice were
361 signaled with a tone indicating strawberry milkshake but punishments were signaled with the
362 house lights, outcome valences were inherently incomparable. The expectation-defined tf-ROI
363 was thus an excellent beginning to the identification of a common reward-specific biomarker;
364 however, the expression of learning-related modulations is only a part of the relevant variance in
365 this reward signal.
366 Expectation-related contrasts are ideal for identifying the specificity of pharmacological
367 manipulations on these reward signals: the significant tf-ROI enhancement of delta power in
368 humans due to d-amphetamine is powerful evidence for a selective and specific enhancing effect
369 of dopamine agonism on this reward-responsive biomarker. Unfortunately, the absence of a
370 learning effect in mice makes the null effect in this contrast less informative. Yet positive findings
371 in the ERP component generation of this signal across both species is still particularly meaningful.
372 An emerging literature is revealing that appetitive motivation and affective value boost the
373 RewP outside any influence of learning or expectation (Threadgill and Gable 2017; Brown and
374 Cavanagh 2018; Peterburs et al. 2019; Huvermann et al. 2021; Pegg et al. 2021). This
375 dissociation in affective vs. informational value might be critical for understanding altered reward
376 dysfunction: for instance, major depression is associated with a diminished RewP but no change
377 in the information representation of positive prediction error, suggesting that mood affects

variance related to the generation of the signal and not the learning-dependent modulation (Cavanagh et al. 2018).

Limitations and Future Directions

Cross-species comparisons in electrophysiological responses are complicated by a number of factors. There are differences between species in cortical homologies and the scale of neural activity in each recording type (i.e. scalp vs. dura). Still, increasing evidence suggests comparable midfrontal cortical structures (Balsters et al. 2020; Schaeffer et al. 2020) and confirms empirical similarities in EEG responses between humans and rodents (Narayanan et al. 2013; Ehlers et al. 2014, 2020; Warren et al. 2015; Featherstone et al. 2018; Robble et al. 2021). There are also inherent differences between species in task experience, motivation, and difficulty. We addressed these methodological difficulties with a highly constrained analytic strategy: we used the same task and the same time-frequency region of interest (tf-ROI) within our 'learn-confirm' strategy between experimental phases (Cavanagh et al. 2021).

While this methodological conservation was designed to constrain interpretation, continued methodological advancements will be required to optimally hone this field of research. For example, our small-scale replication study identified a better technique for enhancing learning (e.g. one 80/20 pair). Our findings reported here only contained a single electrode in humans with a single dura lead in mice. While this theory-driven reduction of spatial dimensionality was appropriate for the constrained methodological approach of this study, it offers only a fraction of assessable EEG activities in each species. In the future, depth recordings might provide more signal to noise than dura screws while simultaneously revealing the source generators of this reward-specific signal.

The use of pharmacological manipulation is a powerful tool for causal inference, although this technique also has inherent limitations. It is important to note that d-amphetamine enhances release of both norepinephrine and serotonin in addition to dopamine, and it is thus conceivable

404 that some of the drug effects detected in this study reflect increases in non-dopaminergic
405 neurotransmission. Yet in contrast to difficult and expensive human clinical trials, future rodent
406 studies using this described paradigm could provide a relatively simple test of the aminergic
407 specificity of the RewP.
408
409 *Summary*
410 This study demonstrated that the RewP is a pharmacologically valid biomarker of reward
411 sensitivity across species. Moreover, we provided a confirmatory test on the role of dopamine in
412 the generation of the RewP. The RewP appears to be a trans-diagnostic biomarker of reward
413 responsiveness, learning, and valuation. The cross-species translatability of this bio-signal will
414 further bolster our mechanistic understanding of reward-related disfunctions in major depression,
415 schizophrenia, and Parkinson's disease.

Data Availability: All data and Matlab code to re-create these analyses are available at OpenNeuro.org, accession #[WILL BE COMPLETED UPON ACCEPTANCE]

Author Contributions (CRediT):
JFC: Conceptualization, Methodology, Software, Formal analysis, Writing - Original Draft, Funding acquisition
SO: Investigation
JAT: Investigation, Data curation, Supervision, Project Administration
JEK: Investigation, Data curation, Supervision, Project Administration
BZR: Investigation, Data curation, Supervision, Project Administration
JAN: Investigation, Data curation, Supervision, Project Administration
JS: Investigation, Data curation, Supervision, Project Administration
DG: Investigation
SGB: Conceptualization, Methodology, Investigation, Data curation, Supervision, Project Administration, Writing - Review & Editing, Funding acquisition
GAL: Conceptualization, Methodology, Resources, Writing - Review & Editing, Funding acquisition
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JWY: Conceptualization, Methodology, Writing - Review & Editing, Funding acquisition
JLB: Conceptualization, Methodology, Resources, Writing - Review & Editing, Funding acquisition

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557 **SUPPLEMENTAL MATERIALS**
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563 **Amphetamine alters an EEG marker of reward processing in humans and mice**
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Age (Mean(SD))	22 (4.82)
Race (%): Caucasian Asian Pacific Islander/Native Hawaiian/Alaskan Mixed race	 39 26 17 15
Gender: M:F	12:11
Education (Mean (SD))	14.26 (1.74)
Smokers: non-smokers	0:23
WRAT score (Mean (SD))	108.13 (11.61)
Caffeine intake in mg/day (Mean (SD))	165 (231.6)
MCCB composite T-score (Mean (SD))	48.17 (9.06)

Supplemental Table 1. Characterization of the human cohort. WRAT: Wide Range Achievement Test; MCCB: MATRICS Comprehensive Cognitive Battery.

Time (hours)	Procedure
0830	Check-in; urine toxicology / pregnancy test; check for medication/lifestyle changes; vital signs & symptom rating scales; standardized breakfast
0900	Pill administration (placebo vs D-amp 10 mg vs. D-amp 20 mg)
0930	Vital signs & symptom rating scales
1055	Vital signs & symptom rating scales
1100	PLT with simultaneous EEG recording
1215	Vital signs & symptom rating scales
1220	Lunch
1315	Vital signs & symptom rating scales
1415	Vital signs & symptom rating scales
1510	Vital signs & symptom rating scales
1610	Vital signs & symptom rating scales
1630	Vital signs & symptom rating scales; checkout

Supplemental Table 2. Timeline for each experimental day for human participants.

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	Main: Drug	Main: Block	Main: Sex	Drug*Block	Drug*Sex	Block*Sex	3-Way
Human	F=3.12 df=55.12 p=.08	**F=32.01** **df=73.74** **p<.001**	F=2.88 df=85.16 p=.09	F=.76 df=94.85 p=.38	**F=5.68** **df=55.12** **p=.02**	F=.74 df=73.74 p=.39	F=1.2 df=94.85 p=.28
Mouse	F=.44 df=141.83 p=.51	F=.45 df=200.47 p=.50	F=1.89 df=192.89 p=.17	F=1.07 df=174.81 p=.30	F=.12 df=141.83 p=.73	F=.44 df=200.47 p=.51	F=.02 df=174.81 p=.89

Supplemental Table 3. Mixed linear model outcomes of behavioral performance accuracy for humans and mice .

Materials and Methods

Animal subjects: pre-training & surgery

All operant behavior was conducted in a custom acrylic chamber measuring 21.6 × 17.8 × 12.7 cm housed within a sound- and light-attenuating box (Med Associates, St. Albans, VT). One end of the chamber contained a house-light, a tone generator, an ultra-sensitive lever, and a magazine with an attached peristaltic pump for delivering milk liquid reward (Nesquik (S.A., Vevey, Switzerland), non-fat powdered milk (Walmart INC., Bentonville, AR USA), and water). The other end of the chamber was fit with a touch-sensitive screen (Conclusive Solutions, Sawbridgeworth, UK) covered by a black acrylic aperture plate allowing two or two 2 × 5 cm touch areas separated by 0.5 cm and located at a height of 6.5 cm from the floor of the chamber. Stimulus presentation in the response windows and touches were controlled and recorded by the K-Limbic Software Package (Conclusive Solutions, Sawbridgeworth, UK).

Prior to training mouse body weights were slowly reduced and then maintained at 85% of their free-feeding body weight. Mice were acclimated to the testing room and the strawberry milk reward by providing 3 drops of reward per mouse on a weigh boat in the home cage for 15 min per day for 3 days. Mice were then habituated to the operant chamber and learned to collect liquid reward from the magazine wherein 30uL of strawberry milk was dispensed every 30 secs after each collection (measured by a head dip into the magazine). Mice that retrieved 10 rewards within 15 min were moved to bar training which required mice to press the lever for reward; criteria was 30 presses in 30 mins. Next mice moved to touch training, which required mice to initiate trials with a bar press and then respond to the presentation of a white (variously shaped) stimulus in 1 of the 2 windows on the touchscreen. The stimulus persisted until a response was made and touches to the blank window had no effect. Touching the stimulus resulted in a 1 second tone and reward delivery. Mice capable of initiating, touching, and retrieving 30 rewards in 30 mins moved to punish training. Punish training was identical to touch training except that touches to the blank window resulted in a 10 sec house light on time-out. Incorrect responses in this stage were followed by correction trials in which the same stimuli and left/right positioning was repeated until a correct response was made. Criteria was set at 75% correct responses in 30 mins.

Mice trained on the three-image set task in the main text next underwent surgery, while mice on the small-scale replication study (Fig. S4) learned to discriminate between two equi-luminescent images. Target images (Fan or Marble) had an 80% probability of reward delivery and a 20% probability of punishment. The non-target image was punished for 80% of trials and rewarded the other 20%. Image-reward contingencies were counterbalanced across mice. Once mice achieved 70% responses to the target image (regardless of rewards or punishments) over 2 days, they were single housed and given 3.0g of chow in preparation for stereotactic surgery.

During surgery, mice were anesthetized with 3% isoflurane and placed in a stereotaxic alignment system (Kopf Instruments, Tujunga, CA, USA) for surgical implantation of dura-resting

skull screws (0.078" stainless steel machine screws (00.90-078-M-SS-PS US Micro Screw Seattle, WA, USA). Screws were placed targeting medial prefrontal cortex (mPFC: AP +2.80, ML +0.00) the primary motor cortex (AP +0.75, ML +1.50), and posterior parietal association cortex (PtA: AP -1.94, ML +1.50) with a cerebellar ground. Silver wire leads soldered to the pins of Omnetics connectors (Omnetics, MN, USA) were wrapped securely around each corresponding screw and secured to the skull using dental cement (Stoelting, IL, USA). After 4 days of recovery, body weight reduction resumed and mice were given post-surgery reminder sessions consisting of the last pre-training regimen to ensure retention of pre-training criterion. Once mice re-attained criteria of 70% target image selection they were moved to tether training which consisted of attaching the recording cable to the electrode cap under light isoflurane 15 mins prior to testing. Here mice learned to respond to stimuli with restricted movement and were required to maintain criteria of 70% target image selection before moving to recording. During each recording session, electrophysiological activity was captured via a multichannel acquisition processor (PlexControl, Plexon, Dallas, TX, USA) at a sample rate of 1000 Hz and behavior automatically video-tracked via headstage-integrated LED to exclude trial irrelevant behavior (CinePlex, Plexon, Dallas, TX, USA). Relevant task events and behaviors were time-coded as event markers on the recording file via TTL pulse from the behavioral software, and were confirmed via video tracking.

Results

Humans: Effect of d-amphetamine on autonomic activity and symptom ratings

D-amphetamine significantly increased heart rate from baseline with the maximum effect from 255 - 370 mins. Main effect of drug $F_{(2,44)}$= 15.5, p<0.0001, time $F_{(6,132)}$= 9.52, p<0.0001 and drug x time $F_{(12, 264)}$ = 3.039, p=0.0005. Post-hoc significant 0-10 mg (p =0.0017) 0-20 mg (p<0.0001) and 10-20 mg (p=0.0351). D-amphetamine significantly increased diastolic blood pressure from baseline with the maximum effect from 115 - 430 mins. Main effect of drug $F_{(2,44)}$= 4.33, p=0.02, time $F_{(6,132)}$= 8.5, p<0.0001 and drug x time $F_{(12,264)}$ = 2.126, p=0.02. Post-hoc significant 0-20 mg (p=0.0052). D-amphetamine significantly increased systolic blood pressure from baseline with the maximum effect at 115 min and then 255 - 430 mins. Main effect of drug $F_{(2,44)}$= 11.22, p=0.0001, time $F_{(6,132)}$= 5, p=0.0001 and drug x time $F_{(12, 264)}$ = 2.328, p=0.007. Post-hoc significant 0-10 mg (p =0.04) 0-20 mg (p<0.0001) and 10-20 mg (p=0.012). There was no effect of d-amphetamine on symptom ratings of anxiety (F<1), happiness (F<1) or drowsiness ($F_{(2,42)}$=1.38,ns), though ratings immediately after EEG testing revealed a significant reduction in drowsiness with d-amphetamine (Main effect of drug $F_{(2,42)=}$3.91, p<0.03; 0 vs. 10 mg: p<0.009). When asked to identify what condition they were in (drug or placebo), participants were 83% correct on placebo days, 65% correct on 10 mg days, and 74% correct on 20 mg days.

Mice: Replication of Simple Expectation Effect

In order to quantitatively inform the emerging literature on this novel bio-signal, we aimed to directly replicate the statistical test from our previous study (Cavanagh et al. 2021). We collapsed the placebo, 0.1 mg/kg, and 0.3 mg/kg conditions and ran a t-test on the tf-ROI delta band power between expectation conditions. Even in the absence of statistically robust learning across the cohort, this contrast was t(27)=1.43, p=.17, *d*=.12, the mean dB difference was .41, and the correlation between this expectation difference dB and aggregate accuracy for each mouse was rho(27)=.16, p=.43. These values are similar to the two cohorts in our foundational paper (*d*'s= .60, .18; dB differences =.65, .65; cohort 1 correlation rho=.38), providing evidence of reproducibility of this expectation-dependent delta-band reward feature.

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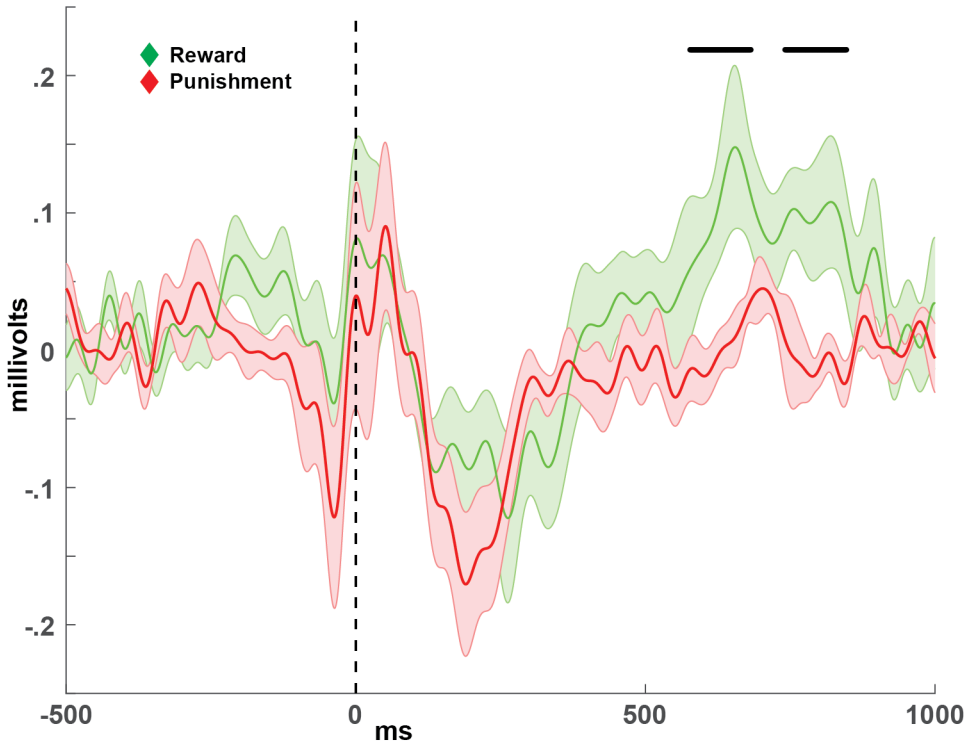

Figure S1. Feedback-locked ERPs from mice in our previous investigation (Cavanagh et al. 2021). Black bars indicate statistically significant differences between conditions (N=12 mice, p<.05, uncorrected for multiple comparisons). The large voltage deflection around time 0 are motion artifacts. Similar to human studies (see discussion), the reward-specific activity differs in time between cohorts. Here the reward-specific activity peaks 500-800ms post-reward.

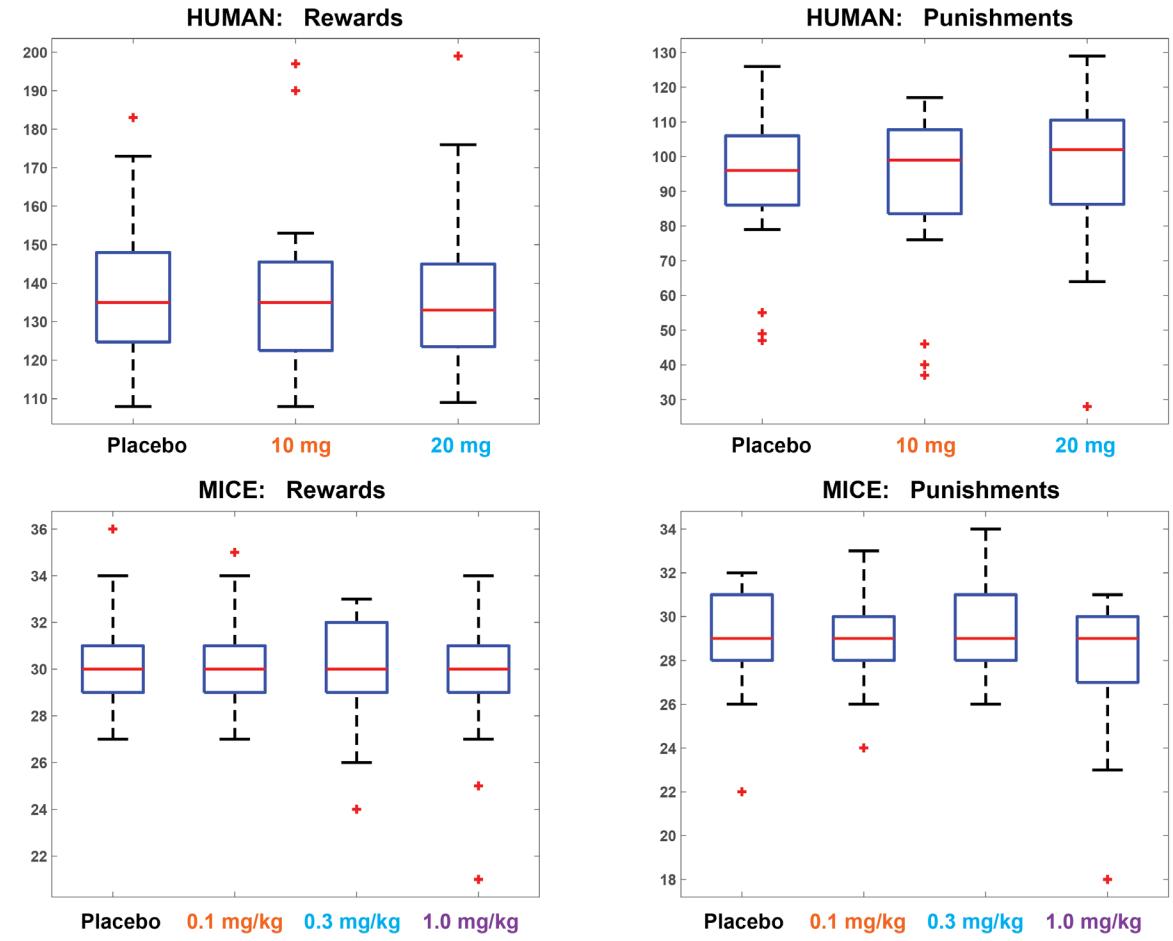

Figure S2. Number of epochs for each condition.

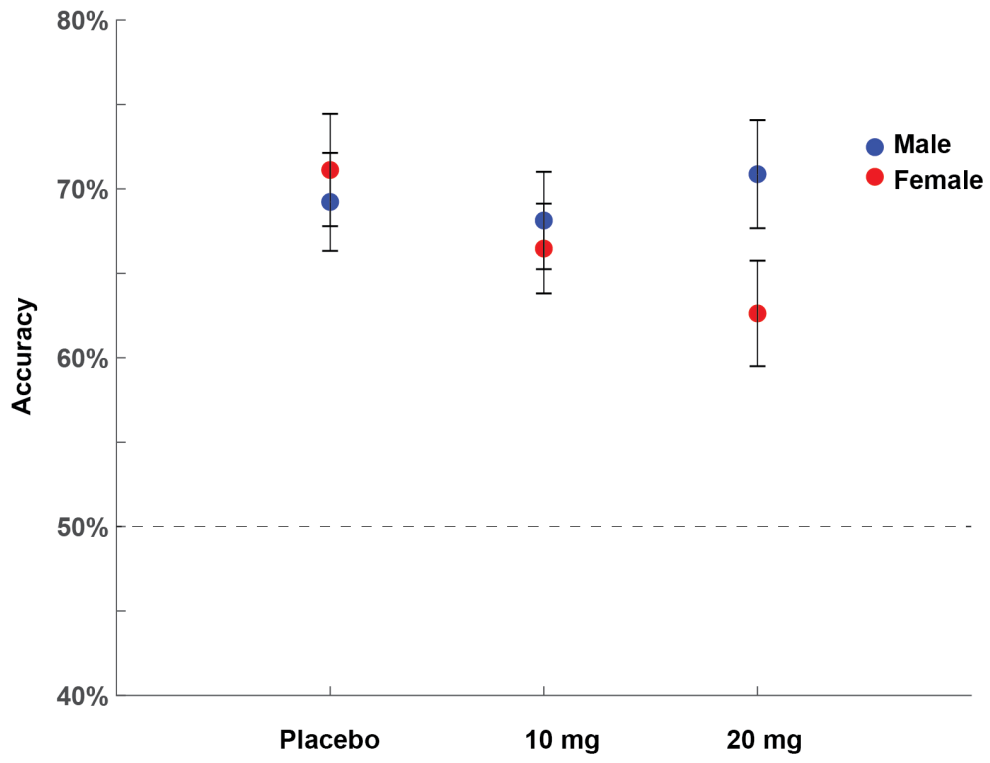

Figure S3. Human behavioral performance collapsed across block. This figure shows the sex*drug interaction whereby increasing dose of d-amphetamine (X-axis) had no effect in males but caused decreased performance accuracy in females.

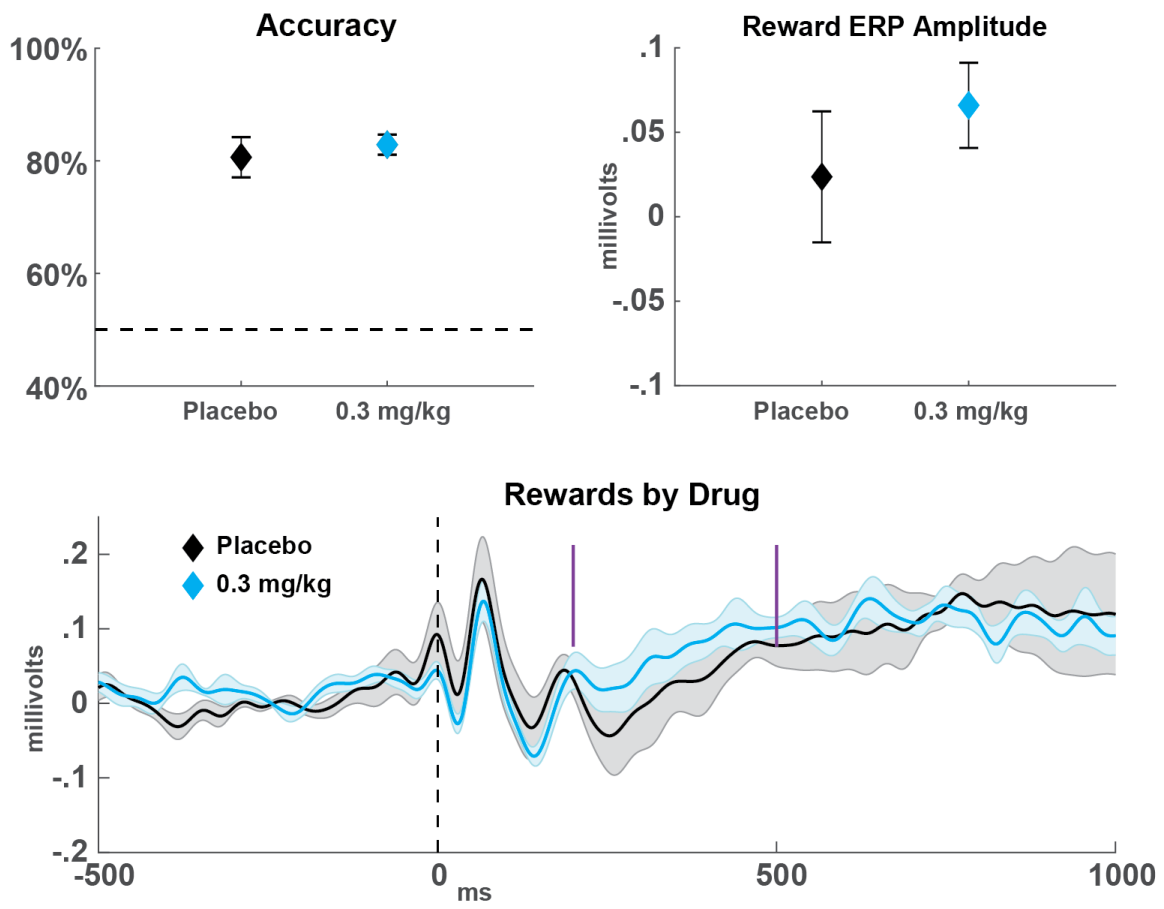

Figure S4. Small-scale replication study. An additional cohort of mice was run with a simpler reward structure and only two drug conditions (placebo, 0.3 mg/kg). All mice were all able to learn to discriminate a single pair of stimuli (target: 80% correct / non-target: 20% correct) at approximate matching levels, with no difference between drug sessions (t(8)=1.03, p=.33). Of these nine mice, six had eight sessions each (four placebo), two had four sessions each (two placebo), and one died after two sessions (one placebo). The reward-specific component peaked earlier than both our prior investigation (Figure S1: 500:800 ms) and the current main text report (Figure 2: 400:600 ms); here the RewP was quantified at 200:500 ms. This analysis showed a trend in the expected direction, but this difference was not statistically significant (t(8)=1.64, p=.14, *d*=.41). However, this contrast has a larger effect size than the paired contrast for these two conditions from the main text: t(27)=2.02, p=.05, *d*=.23. In sum, these findings suggest that these ERP and amphetamine effects are reliable, but like in human ERP findings there is substantial inter-individual variability that affects component identification and statistical power. All plots are mean +/- SEM.

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