Amphetamine alters the Reward Positivity in humans and mice

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Abstract

The development of pro-cognitive therapeutics for psychiatric disorders has been beset with difficulties. 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 measure 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. Here, we determined the impact on this reward-related EEG response from humans (n=23) and mice (n=28) performing a probabilistic learning task under parametric doses of d-amphetamine (human: placebo, 10 mg, 20 mg; mice: placebo, 0.1 mg/kg, 0.3 mg.kg, 1.0 mg/kg). In humans, d-amphetamine boosted the Reward Positivity event-related potential (ERP) component as well as the spectral delta-band representation of this signal. In mice, only the Reward Positivity ERP component was significantly boosted by d-amphetamine. In sum, the present results confirm the role of dopamine in the generation of the Reward Positivity, and support the first pharmacologically valid biomarker of reward sensitivity across species.

Introduction

Animal models of cognitive processes have been beset by translational difficulties. We recently addressed this problem by identifying common electrophysiological biomarkers of isolated cognitive processes in both humans and mice[1]. One of the most promising outcomes identified in that project was a common delta-band spectral reflection of the *Reward Positivity* (RewP), an event-related potential (ERP) component that has been well-described in human participants[2,3]. While the discovery of cross-species biomarkers is an important step, their translational utility requires a demonstration of consistent pharmacological predictive validity (i.e. similar drug effects across species). Here, we tested whether this cortical reward signal had similar sensitivity to d-amphetamine in mice and humans.

The RewP occurs as a positive deflection in the ERP that is most commonly quantified over fronto-central sites around 200-400ms after reward presentation [2,4,5]. Both the RewP and its delta-band spectral reflection scale with the degree of positive reward prediction error, whereby "better than expected" outcomes evoke increasingly larger RewP amplitudes [6–8]. These two criteria are notable: they indicate that this reward-related signal is specific and sensitive to positive reward prediction error, fulfilling the stringent criteria of being an invariant neural marker of this computational process[9].

The RewP is diminished in disorders like major depression[10,11] and Parkinson's disease[12], suggesting it might be a trans-diagnostic biomarker of reward responsiveness, learning, and valuation. The emerging mechanistic understanding of this signal increases its appeal: the reward prediction error computation reflected by the RewP mirrors the dopamine-driven learning process underlying reinforcement learning[13]. The cortically-generated RewP has been theorized to be modulated by phasic midbrain dopamine[3], however empirical tests of this have been sparse and beset by methodological complications (see discussion), with little evidence for pharmacological predictive validity across species. It remains unknown if these cortical and midbrain systems influence each other in a causal manner, if they are both influenced

81 by a third variable, or if they reflect parallel processes. Despite these uncertainties, the current
82 understanding of the RewP suggests that it should be affected by manipulations of dopaminergic
83 activity. The current study aimed to provide an initial test of this intuitive hypothesis, as well as
84 facilitate the first test of common underlying mechanisms in the homologous mouse cortical
85 signal.
86

87 **Materials and Methods**

88 *Human Participants*

89 Healthy women and men between the ages 18-35 years were recruited from the
90 community via public advertisements and compensated monetarily for study participation. This
91 study was conducted at the UCSD Medical Center and was approved by the UCSD Human
92 Subject Institutional Review Board. Participants first completed a phone screen to assess current
93 and past medical and psychiatric history, medication and recreational drug use and family history
94 of psychosis. During the subsequent in-person screening visit, participants signed the consent
95 form and completed assessments of physical and mental health, including a structured clinical
96 interview, self-report questionnaires about caffeine intake and handedness, a hearing test,
97 physical examination, an electrocardiogram, urine toxicology screen, urine pregnancy test for
98 females, the MATRICS Comprehensive Cognitive Battery (MCCB) and a Wide Range
99 Achievement Test (WRAT) for IQ assessment. See Supplemental Table 1 for these clinical
100 demographics. A total of 12 male and 11 female participants completed all three sessions and
101 are included here.

102 A double blind, randomized, placebo-controlled, counterbalanced, within-subject design
103 was utilized. Participants received either placebo or one of two active doses of d-amphetamine
104 (10 or 20 mg) orally on each of the three test days which were separated by one week. On all
105 assessment days, participants arrived at 8:30 am after overnight fasting, completed a urine
106 toxicology screen and a urine pregnancy test in females, and ate a standardized breakfast. Vital

signs and subjective symptom rating scale scores were obtained at specific intervals pre- and post- administration. Starting 120 minutes post-administration, subjects completed cognitive tests and simultaneous EEG recording. See Supplemental Table 2 for experimental session information.

Probabilistic Learning Task (PLT)

This version of the PLT was identical to our previous investigation[1], see Figure 1A. Participants were presented a stimulus pair (e.g., bicycle/phone, chair/clip, plug/flashlight) on a computer monitor and instructed to select the “target” stimulus using a digital 4-switch USB arcade-style joystick. Participants were given feedback after each trial about whether their response was “correct” or “incorrect”. Reward probabilities for the target / non-target stimulus were set within a block of 80 trials (80/20, 70/30, 60/40, 50/50), but stimuli differed between trial blocks (first block was bicycle/phone at 80/20, then the next block was chair/clip at 60/40, etc.). Overall performance was calculated as the total number of correct target selections in each block of 80 trials.

Human Electrophysiological Recording and Pre-Processing

Continuous electrophysiological (EEG) data were recorded in DC mode from 64 scalp leads using a BioSemi Active Two system. Four electrooculograms (EOG) recorded at the superior and inferior orbit of the left eye and outer canthi of each eye, and one nose and two mastoid electrodes were also collected. All data were collected using a 1048 Hz sampling rate utilizing a first-order anti-aliasing filter. Custom Matlab scripts and EEGLab [14] functions were used for all data processing. Data were first down sampled to 500 Hz, epoched around the imperative cues (-2000 to +5000 ms) and then average referenced. Bad channels and bad

130 epochs were identified and were subsequently interpolated and rejected respectively, then blinks
131 were removed following independent component analysis.
132
133 *Animal Subjects*
134 Female and male C57BL/6J mice were obtained from The Jackson Laboratory (Bar
135 Harbor, ME), housed in same sex groupings of 2 per cage in a temperature- and humidity-
136 controlled vivarium under a reverse 12 h light/dark cycle (lights off 0800 h) and tested during the
137 dark phase. All experimental procedures were performed in accordance with the National
138 Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the
139 University of New Mexico Health Sciences Center Institutional Animal Care and Use Committee.
140 A total of 15 male and 13 female mice were used. See Supplemental Methods for information on
141 touchscreen pre-training and surgery.
142 A randomized, placebo-controlled, counterbalanced, within-subject design was utilized.
143 Mice received either placebo or one of three active doses of d-amphetamine (0.1 mg/kg, 0.3
144 mg/kg, 1.0 mg/kg) on each of the four test days which were separated by a 72 hours washout
145 period. Starting 15 minutes post-administration, subjects completed the PLT with simultaneous
146 EEG recording. Notably, since 1.0 mg/kg leads to significantly increased hyperactivity and
147 significantly decreased task focus, it was investigated as an upper limit of dosing.
148
149 *Mouse PLT*
150 This version of the PLT was also identical to our previous investigation[1], see Figure 1A.
151 Each session, mice were presented with three pairs of unique stimuli (fan/marble, honey/cave,
152 spider/fan) in three separate 20 trial blocks. For the first block, one stimulus was rewarded 90%
153 of the time and the other was rewarded 10% of the time. The next blocks included 80/20 and then
154 70/30 reinforcement rates. The mice were given 120 minutes to complete the task. Rewards
155 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. A 20 Hz low pass filter was applied to all ERPs. 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[1], 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[1]. ERPs were not reported in our prior 'learn phase' investigation; we show the relevant mouse ERPs here in

182 Supplemental Figure 1. We also report additional analyses that were facilitated by this novel
183 'confirm'-stage multi-session, within subject design. As in our previous report, hypotheses were
184 specific to the reward-related EEG; however, punishment-related EEG signals are again reported
185 here for consistency.

186 Species were analyzed with separate mixed effects models. Each mouse was treated as
187 a random effect, similar to humans. Drug condition and sex were treated as fixed effects, although
188 there were no *a priori* hypotheses for sex effects. Given the established effects of dopamine
189 agonism on frontal cortical activities, either linear or quadratic effects could be expected
190 (depending on dose and individual differences), so the trend was analyzed based on the visual
191 depiction of outcomes. Fitting with the demands of parsimony and the inferential affordance
192 allowed by the number of drug conditions, this resulted in linear contrasts for humans (placebo,
193 10 mg, 20 mg) but quadratic contrasts for mice (placebo, 0.1 mg/kg, 0.3 mg/kg, 1.0 mg/kg).

194 Scalar values of spectral power were derived from the same spatial, temporal, and
195 frequency windows as in our foundational paper. For these expectation-based contrasts,
196 comparisons were split based on the probabilistic aspect of the reward feedback, creating high
197 probability (i.e. target response followed by reward) vs. low probability (i.e. non-target response
198 followed by reward) contrasts. We adhered to our previously-defined tf-ROIs and we again
199 omitted data from the 50/50 condition in humans (human reward: 1.3 Hz to 2 Hz, 250 to 550 ms;
200 human punishment: 4 to 6.5 Hz, 450 to 750 ms; mouse reward: 1 to 4 Hz, 250 to 550 ms; mouse
201 punishment: 4.5 to 7.5 Hz, 500 to 800 ms). However, this study design offered an additional
202 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)[2,15–18], highlighting how learning is not a necessary requirement for eliciting these cortical signatures of feedback receipt.

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: 250 to 400 ms, human punishment: 250 to 500 ms. mouse feedback: 400 to 600 ms), see Figure 2. Mouse ERP quantifications had high skewness so each drug condition was winnowed to delete outlying values until skewness<1 (4-6 values deleted per condition).

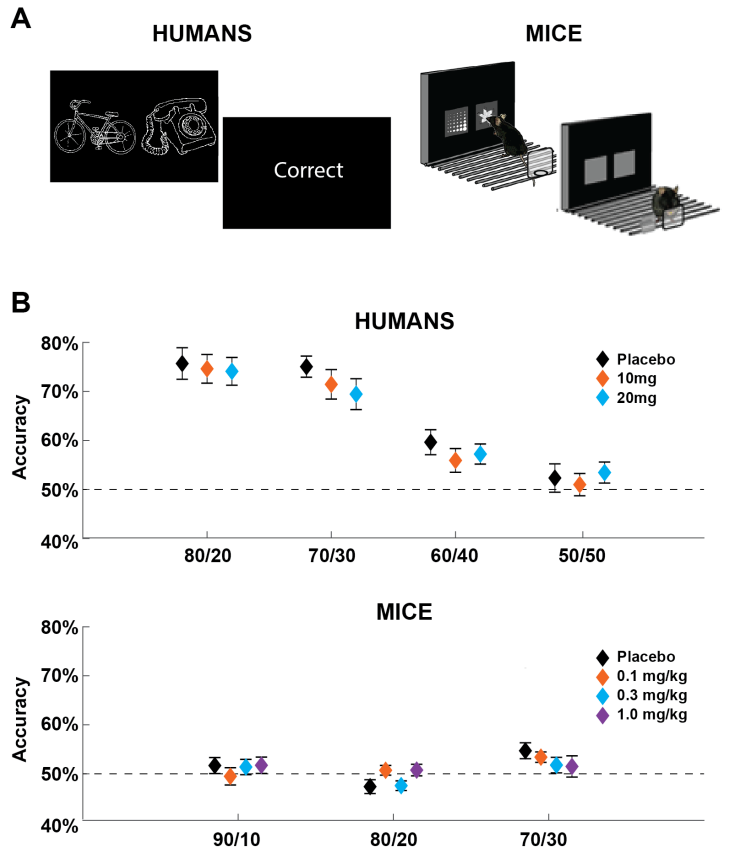

Figure 1. Task and performance. A) The Probabilistic Learning Task required the subject to select the stimulus that probabilistically led to reward most often. In humans and mice, each trial required a choice between two stimulus icons. B) Performance, split by block and drug. Humans displayed approximate 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.

229 In humans the grand average time-frequency activity peaked earlier than the expectation-defined
230 contrasts, so we defined new time ranges based on the grand average over all drug conditions.
231 For reward, this earlier window was from 100 to 400 ms and for punishment this earlier window
232 was 250 to 550 ms. There was no apparent simple grand average peak for feedback in mice, so
233 the previously defined tf-ROIs were used in all analyses.
234
235 **Results**
236 *Performance*
237 All statistical results for performance data are reported in Supplemental Table 3. In
238 *humans*, there was a main effect of block (easier pairs were more accurate, Figure 1B) and a
239 drug*sex interaction, which revealed a tendency for females to perform slightly worse under
240 higher amounts of d-amphetamine whereas males were unaffected (see Supplemental Fig 2). In
241 *mice*, there was no effect of block nor any effect with drug. This indicates that mice had poor
242 performance discriminating the probabilities between difficulty conditions, although they managed
243 to perform above chance ($t(27)=2.15$, $p=.04$), see Figure 1B.
244
245 *ERPs*
246 All statistical results for ERP data are reported in Table 1. In *humans*, there was a main
247 effect of drug with a linear trend for increased RewP amplitude with increasing dose of d-
248 amphetamine. This effect was not present in the corresponding punishment condition, which
249 showed a trend towards a quadratic effect (although the quadratic contrast was not significant
250 either). In *mice*, there was a quadratic effect of drug on reward ERP amplitude, with no effect in
251 the punishment condition. Since 1.0 mg/kg of d-amphetamine was at ceiling-level tolerance and
252 is thus not ideally suitable for comparison to humans, we examined if the same linear effect
253 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, again only for reward ERP component amplitude.

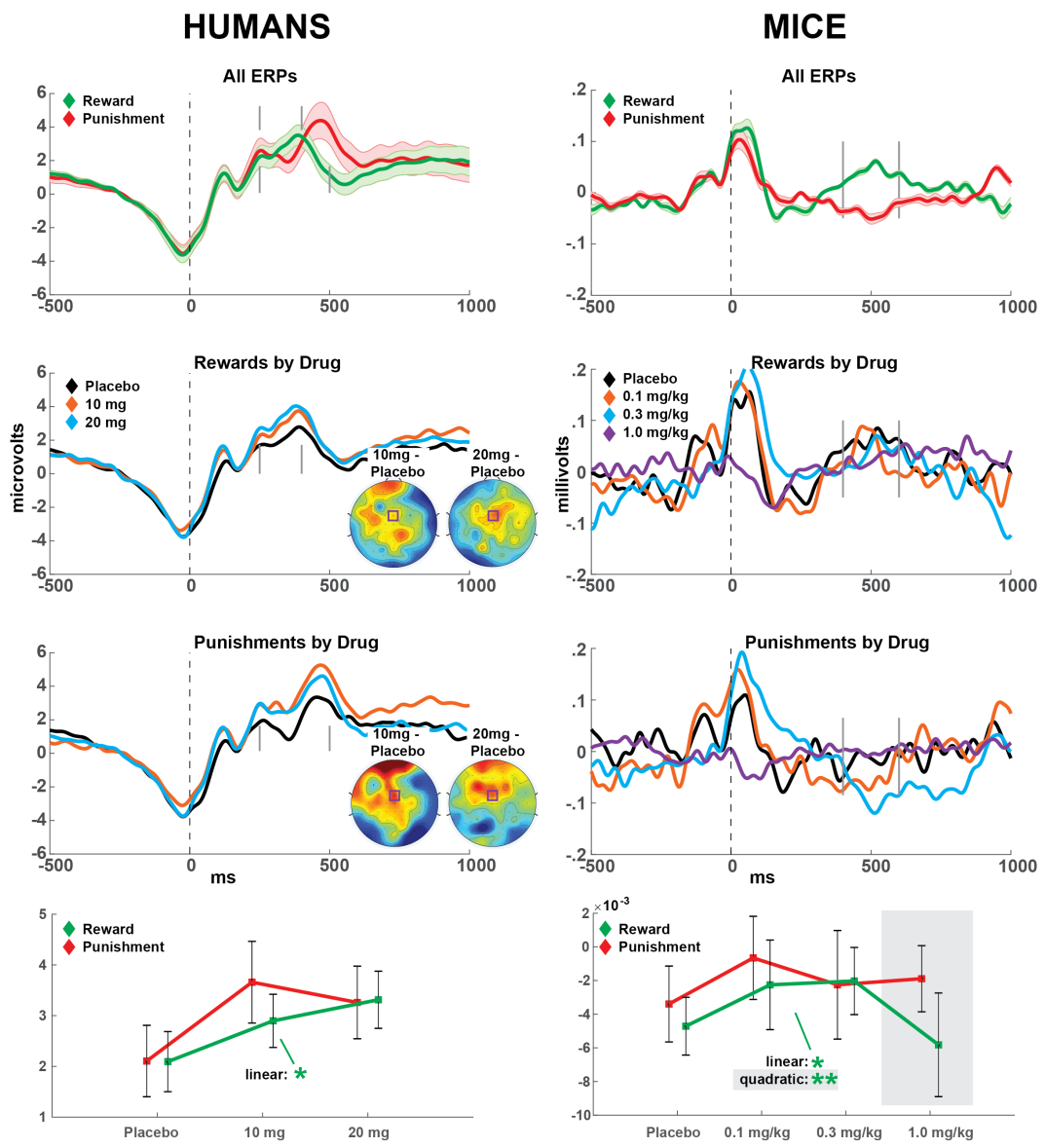

Figure 2. ERPs. Grand average plots show feedback-locked ERP activity averaged over all drug conditions. Time windows for quantification are show with grey bars. Below, each feedback condition ERP is shown, split by drug dose. The same time window quantifications are shown with grey bars. Human ERPs 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. Only reward ERP components were significantly affected by d-amphetamine. There was a quadratic effect in mice, 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=1.42 df=47.53 p=.24	F=1.48 df=34.85 p=.23	F=1.16 df=47.07 p=.29	**F=8.81 df=48.49 p=.005**	F=3.86 df=48.44 p=.06	**F=5.00 df=78.98 p=.03**
	tf-ROI Grand Average	F=1.16 df=49.46 p=.29	F=.87 df=60.29 p=.35	F=2.93 df=49.46 p=.09	F=1.16 df=75.02 p=.29	F=.02 df=75.02 p=.89	F=1.02 df=26.83 p=.32
	tf-ROI Expectation Contrast	F=1.05 df=76.70 p=.31	F=.53 df=60.17 p=.47	F=.50 df=76.70 p=.48	F=2.38 df=105.30 p=.13	F=1.22 df=105.30 p=.27	F=.02 df=53.91 p=.89
Mouse: Punishment	ERP	F=.02 df=41.48 p=.90	F=.83 df=40.41 p=.37	F=.35 df=41.49 p=.56	F=.004 df=36.69 p=.95	F=.24 df=36.69 p=.63	F=1.51 df=45.08 p=.23
	tf-ROI Grand Average	F=.07 df=74.40 p=.79	F=1.88 df=54.21 p=.18	F=.06 df=74.40 p=.81	F=.02 df=58.88 p=.88	F=.68 df=58.88 p=.41	F=.07 df=38.79 p=.80
	tf-ROI Expectation Contrast	F=2.98 df=64.80 p=.09	F=.09 df=55.40 p=.77	F=.23 df=64.80 p=.63	F=.28 df=70.96 p=.60	F=.003 df=70.96 p=.96	**F=7.08 df=32.84 p=.01**

Table 1. Mixed linear model outcomes for ERP as well as grand average 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

260 theta power with increasing dose of d-amphetamine, see Figure 3. For reward, there was also a
261 main effect of sex where males had higher reward delta power. In *mice*, there were no significant
262 outcomes for either reward or punishment.
263
264 *TF-ROI: Expectation*
265 All statistical results for tf-ROI expectation contrasts (low probability minus high
266 probability) are reported in Table 1. In *humans*, there were main effects of drug with a linear
267 trend for increased reward delta power with increasing dose of d-amphetamine. This effect was
268 not present in the corresponding punishment condition. There was also a main effect of sex
269 (higher power in males) and an interaction between drug and sex, where this expectation
270 difference declined slightly in males and increased in females with increasing dose of d-
271 amphetamine. In *mice*, the only effect was a linear trend for punishment theta after the removal
272 of the 1.0 mg/kg condition, however this is likely due to removal of sex as a factor and recovery
273 of a degree of freedom which boosted the nearly significant main effect of drug to statistical
274 significance. In sum, only humans displayed an effect of d-amphetamine on reward-related tf-
275 ROI power.
276
277 *Small-scale replication of mouse ERP findings*
278 We further aimed to replicate these appealing mouse ERP findings in a separate small-
279 scale cohort with easier learning discriminations (a single pair of stimuli: fan 80% correct vs.
280 marble 20% correct). Nine mice were run on placebo or 0.3 mg/kg of d-amphetamine across
281 multiple sessions; all learned to perform at 70% accuracy with no difference between drug

conditions. Supplemental Figure S3 shows that this drug-related enhancement of ERP amplitude could be replicated, although it did not achieve statistical significance (t(8)=1.64, p=.14).

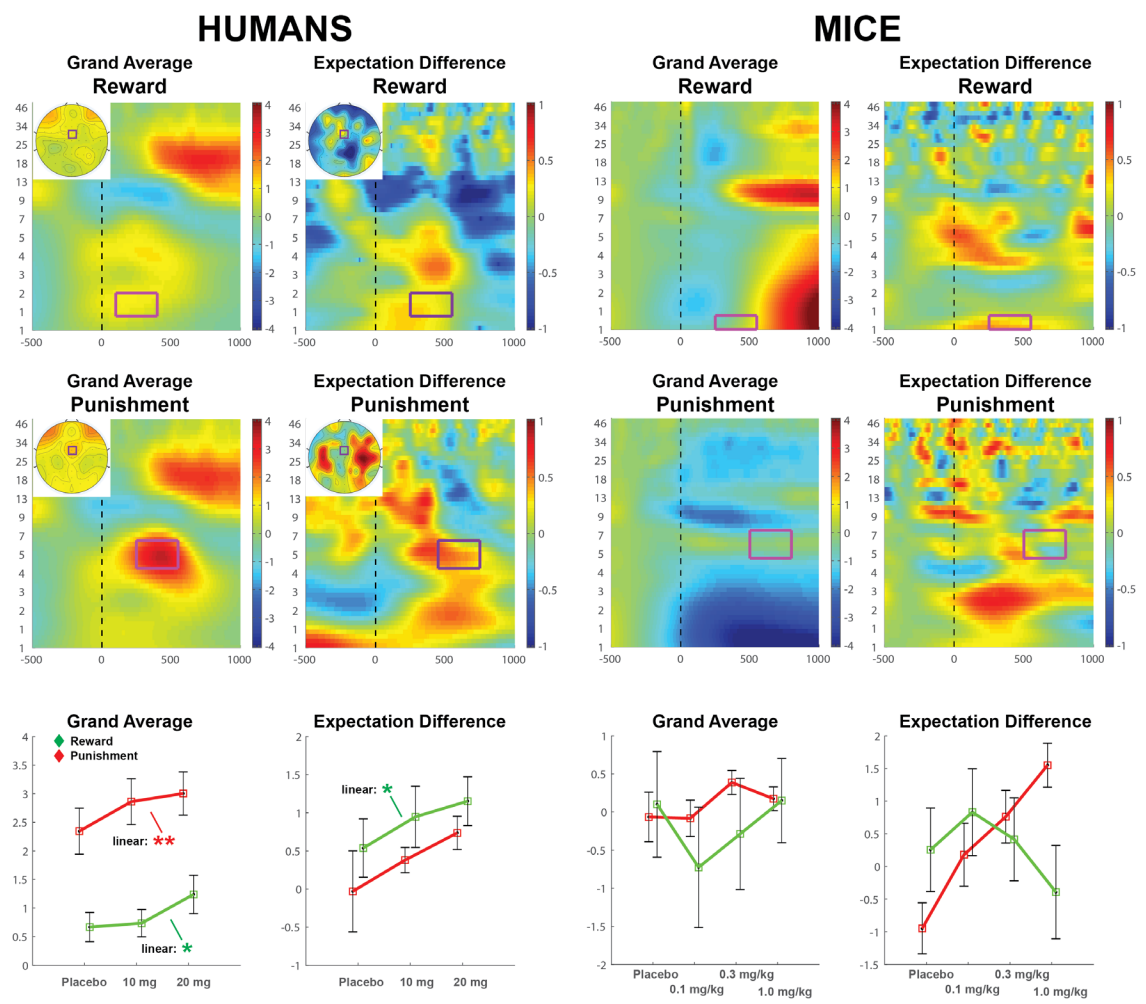

Figure 3. Time-Frequency grand averages and expectation-related contrasts. Time-frequency plots of grand average and expectation difference (feedback from low vs. high probability conditions) at FCz in humans or the anterior lead in mice. The magenta box shows the tf-ROI. Human figures: grand average 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[1]. The current work

288 went further however, by demonstrating similar sensitivity of both species to increasing doses of
289 d-amphetamine, demonstrating pharmacological predictive validity for this biomarker of reward
290 sensitivity. While our previous cortical marker was based on the expectation-modulated spectral
291 power, this study only found common cross-species amphetamine effects in the grand average
292 ERP component. This specificity suggests that the influence of amphetamine is intrinsic to the
293 generation of the RewP and is not *dependent* on experience-relevant modulation.
294
295 *Common d-Amphetamine Effects Across Species*
296 As noted earlier, the RewP is sensitive to learning-related prediction errors, yet it is elicited
297 by any rewarding feedback even in un-learnable environments [2,15–18]. This tendency appears
298 to be preserved in the mice, who performed above chance but not at a level indicative of active
299 successful learning. Our second small-scale replication cohort with good performing mice
300 suggests that the enhancing effect of d-amphetamine on the RewP is reliable, but future
301 experiments should aim to boost statistical power with methodological variations. In particular,
302 depth recordings might provide more signal to noise than dura screws while simultaneously
303 revealing the source generators of this reward-specific signal, in addition to identifying local
304 generators of the RewP signal.
305 While there may have been general facilitating effects of d-amphetamine on human EEG
306 amplitudes, the cumulative cross-species similarities of d-amphetamine administration were
307 strongest for the reward-related grand averages. For instance, whereas the reward-related ERP,
308 grand average tf-ROI, and expectation tf-ROI were all significantly affected by d-amphetamine in
309 humans, punishment-related effects were more mixed and had some different overall trends than
310 the RewP. The effect of d-amphetamine on mouse feedback was even more differentiated
311 between outcome valences, with effects only for the RewP ERP. Why did d-amphetamine affect
312 spectral power in humans but not mice? One reason might be the lower peak frequency of the

mouse delta band phenomenon, which makes effective time-frequency quantification more difficult.

Reward Expectation

Our previous investigation of cortical feedback signals was limited by the need to create a well-controlled analytic contrast within each species (i.e. low vs. high probability outcomes corresponding to high vs. low reinforcement prediction error), without interference from different sensory or imperative stimuli[1]. Since rewarded actions for mice were signaled with a tone indicating strawberry milkshake but punishments were signaled with the house lights, outcome valences were inherently incomparable. Even in humans, conceptual issues about different generative systems underlying reward and punishment EEG signals casts doubt on the suitability of this common “difference wave” contrast [18,19]. The expectation-defined tf-ROI was thus an excellent beginning to the identification of a common reward-specific biomarker, but for methodological and theoretical reasons future use of this marker should not be constrained by seminal limitations. First, even within the well-detailed human literature, the time, space, and frequency of the RewP vary considerably [7,10,20,21]. Second, the expression of expectation differences is only a part of the relevant variance in the grand average RewP signal in humans. An emerging literature is revealing that appetitive motivation and affective value boost the RewP[22–26]. This dissociation might be critical for understanding altered reward dysfunction: for instance, major depression is associated with a diminished RewP but no change in the information representation of positive prediction error, suggesting that mood affects variance

334 related to the generation of the signal (e.g. value) and not the learning-dependent modulation
335 [21].
336
337 *Dopaminergic effects on the RewP*
338 Previous evidence of dopaminergic sensitivity of these cortical signals in humans has
339 been mixed, although likely largely due to methodological reasons. Some studies have examined
340 pharmacological effects on the punishment-related EEG signal or the difference between reward
341 and punishment conditions, and have generally failed to find effects of the dopamine D2 receptor
342 antagonists haloperidol [27] or sulpiride [28,29] unless moderated by genotype [30]. However, as
343 we detailed earlier, differences in the generative systems underlying the reward and punishment
344 signals render the investigation of these combined signals less useful. One experiment revealed
345 a reduction in the RewP in response to the dopamine D2/3 receptor agonist pramipexole [31], yet
346 there are no other studies of parametric or enhancing effects of dopaminergic agents specific to
347 the RewP in humans. A recent report observed that although the RewP is specifically diminished
348 in Parkinson's disease, L-dopa administration did not alter this signal [32]. This surprising lack of
349 acute dopaminergic influence might be due to combined issues of cortical degeneration in
350 Parkinson's and the differential influence of L-dopa on striatal vs. cortical dopamine tone[33]. It
351 is important to note that d-amphetamine enhances release of both norepinephrine and serotonin,
352 in addition to dopamine, and it is thus conceivable that some of the drug effects detected in this
353 study reflect increases in non-dopaminergic neurotransmission. In contrast to difficult and
354 expensive human clinical trials, future rodent studies using this described paradigm could provide
355 a relatively simple test of the aminergic specificity of the RewP.
356
357 *Summary*
358 This study demonstrated that the RewP is a pharmacologically valid biomarker of reward
359 sensitivity across species. Moreover, we provided a confirmatory test on the role of dopamine in

360 the generation of the RewP. The RewP appears to be a trans-diagnostic biomarker of reward
361 responsiveness, learning, and valuation. The cross-species translatability of this bio-signal will
362 further bolster our mechanistic understanding of reward-related disfunctions in major depression,
363 schizophrenia, and Parkinson's disease.

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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
NRS: Conceptualization, Methodology, Writing - Review & Editing, Investigation, Data curation, Supervision, Project Administration, Funding acquisition
JWY: Conceptualization, Methodology, Writing - Review & Editing, Funding acquisition
JLB: Conceptualization, Methodology, Resources, Writing - Review & Editing, Funding acquisition

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Amphetamine alters the Reward Positivity in humans and mice

SUPPLEMENTAL MATERIALS

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

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 liquid dispenser delivering strawberry milk liquid reward (Nesquik (S.A., Vevey, Switzerland), Carnation powdered milk (Nestle Baking, a division of Nestle USA, Inc, Solon, OH 44139 USA), and water) into a magazine. 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 five active touch areas measuring 2.5 × 2.5 cm separated by 0.6 cm and located at a height of 1.6 cm from the floor. Stimulus presentation in the response windows and touches were controlled and recorded by the K-Limbic Software Package (Conclusive Solutions, Sawbridgeworth, UK).

The weights of mice were first slowly reduced and then maintained at 85% free-feeding body weight. Prior to training, mice were acclimated to the strawberry milk reward by providing 3 mL of reward per mouse on a weigh boat in the home cage for 3 days. After becoming acclimated to the reward, mice were habituated to the operant chamber and the magazine with 30 min sessions where 40uL of strawberry milk was dispensed 60 seconds after each collection (measured by a head dip into the magazine). Mice that retrieved 10 rewards within 15 min were moved to touch training. A session during training consisted of 30 total trials or 30 min, whichever was achieved first. Touch training required mice to first initiate each trial with a nose poke into the magazine, followed by a 4 sec ITI, and then respond to a presentation of a white square stimulus in 1 of the 5 response windows (spatially pseudorandomized). The stimulus remained on the screen until a response was made to the stimulus. These responses resulted in reward presentation, a 1 sec tone, and illumination of the magazine.

Once the mouse finished drinking and withdrew, the magazine light was re-illuminated for initiation of the next trial. Mice that retrieved 30 rewards within 30 min were moved to “punish” training where incorrect responses resulted in a 10 second time out with the house light on to discourage indiscriminate responding. Mice could also respond during the ITI, but this was discouraged with a 10 second time out with the house light on. Mice that initiated 30 touches within 30 minutes were then moved to standard training. In this phase, the stimulus remained on the screen for a limited amount of time followed by a 2 sec hold where the touch windows remained active. After completion of the final step mice were given *ad lib* food for three days prior to surgery.

In surgery, mice were anesthetized with isoflurane and placed in a stereotaxic alignment system (Kopf Instruments, Tujunga, CA) for fitting with dura-resting leads. Skull screws were 0.078” stainless steel machine screws (00.90-078-M-SS-PS US Micro Screw) that were placed targeting medial prefrontal cortex (mPFC: AP +2.80, ML +0.00) and posterior parietal association

cortex (PtA: AP -1.46, ML +1.00) with a cerebellar ground. Silver wire leads soldered to the pins of Omnetics connectors wrapped securely around each corresponding screw and secured to the skull using dental cement. After 7 days of recovery, body weight reduction resumed and mice were given a post-surgery reminder session consisting of the last pre-training regimen to ensure retention of pre-training criterion. During each recording session, electrophysiological activity was captured via a multichannel acquisition processor (PlexControl, Plexon) at a sample rate of 1000 Hz and behavior automatically video-tracked via headstage-integrated LED to exclude trial irrelevant behavior (CinePlex, Plexon). 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[1]. We collapsed the placebo, 0.1, and 0.3 mg/kg conditions and ran a t-test on the tf-ROI delta band power between expectation conditions. This contrast was t(27)=1.92, p=.07, *d*=.16, the mean dB difference was .56, and the correlation between this expectation difference dB and aggregate accuracy for each mouse was r(27)=.17, p=.39. These values are highly 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 rigor and reproducibility of this delta-band phenomenon across species.

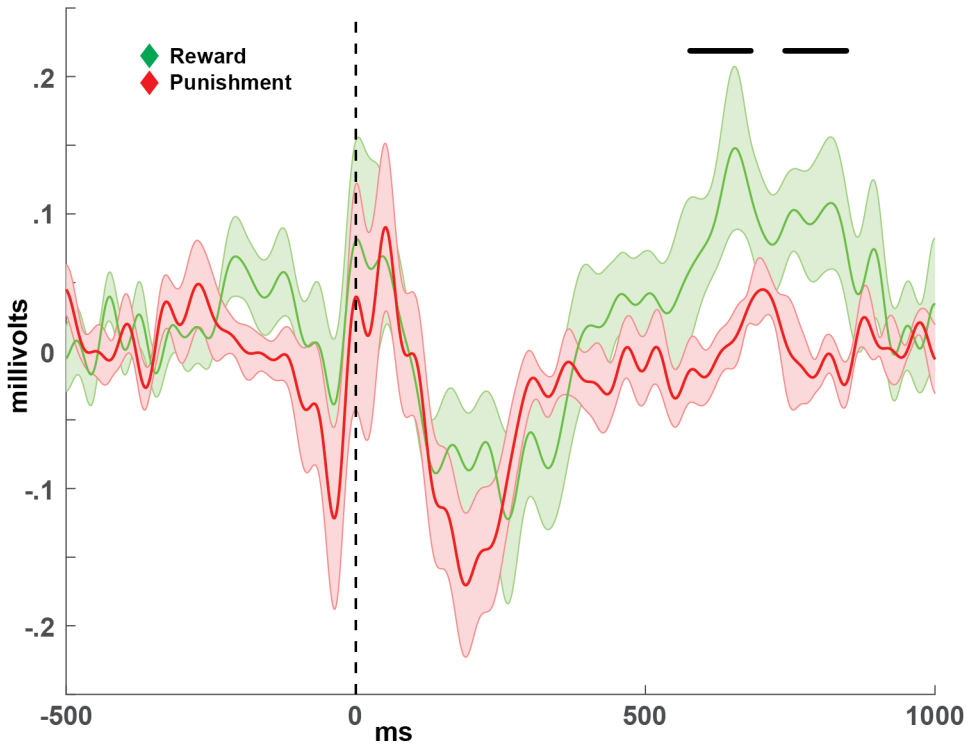

Figure S1. Feedback-locked ERPs from mice in our previous investigation. Black bars indicate statistically significant differences between conditions (N=12 mice, p<.05, uncorrected for multiple comparisons). The large voltage deflection around time 0 are motion artifacts. Similar to human studies (see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

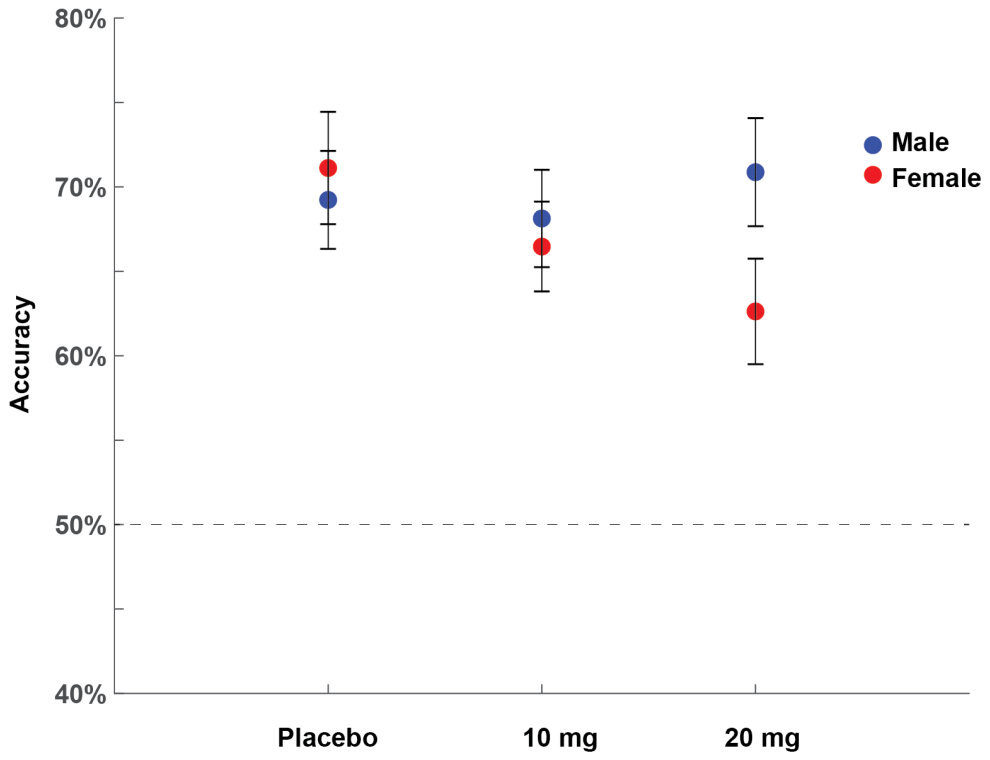

Figure S2. Human behavioral performance collapsed across block. This figure shows the sex*drug interaction whereby increasing dose of d-amphetamine (X-axis) had no effect in males but caused decreasing performance accuracy in females.

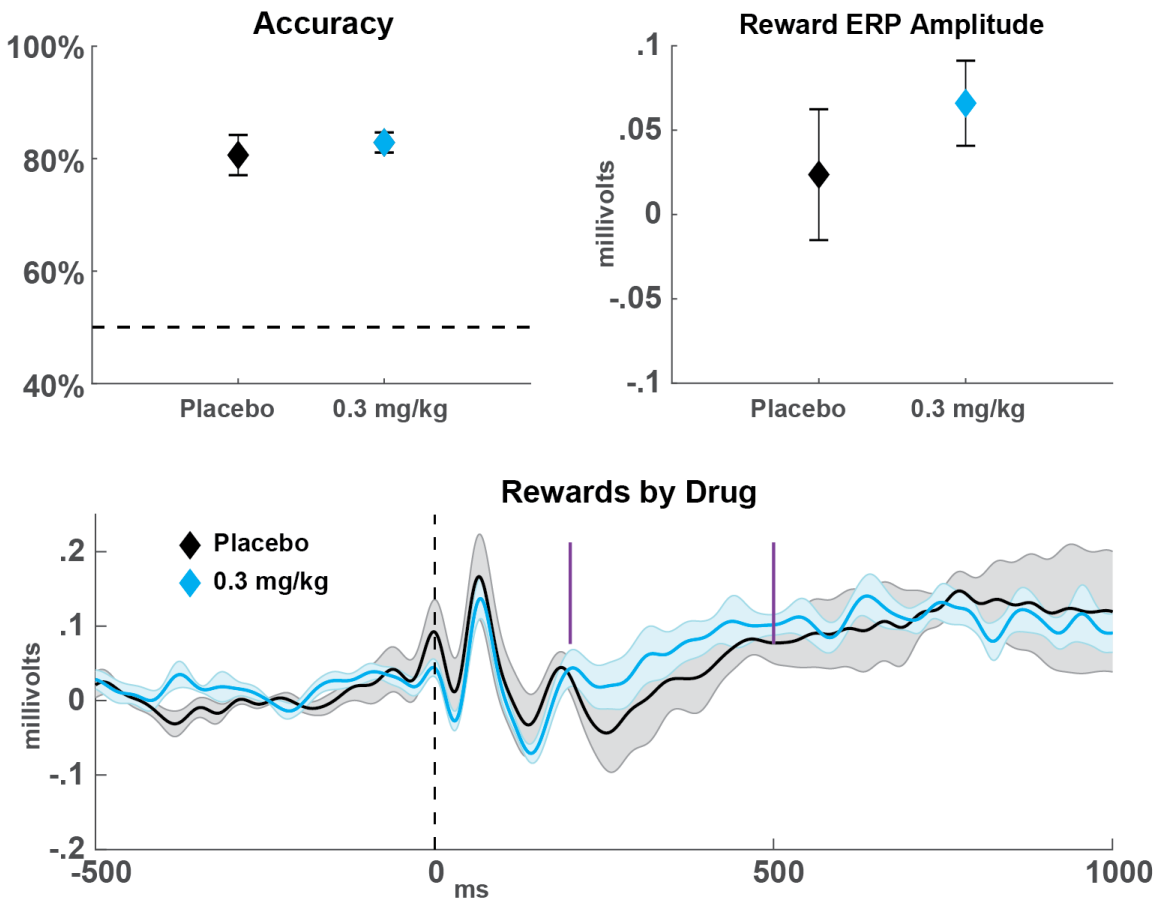

Figure S3. Small-scale replication study. An additional cohort of mice was run with a simpler reward structure and only two drug conditions (placebo, 0.3 mg/kg). All mice were all able to learn to discriminate a single pair of stimuli (fan: 80% correct / marble: 20 % correct) at approximate matching 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 RewP 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). However, this contrast is stronger than the paired contrast for these two conditions from the main text: t(19)=1.23, p=.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 statistical power. All plots are mean +/- SEM.

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