1 **Effects of access condition on substance use disorder-like phenotypes in male and**
2 **female rats self-administering MDPV or cocaine**

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27 **Abstract**

28 Substance use disorder (SUD) is a heterogeneous disorder, where severity, symptoms,
29 and patterns of substance use vary across individuals. Yet, when rats are allowed to self-
30 administer drugs such as cocaine under short-access conditions, their behavior tends to
31 be well-regulated and homogeneous in nature; though individual differences can emerge
32 when rats are provided long- or intermittent-access to cocaine. In contrast to cocaine,
33 significant individual differences emerge when rats are allowed to self-administer 3,4-
34 methylenedioxypyrovalerone (MDPV), even under short-access conditions, wherein
35 ~30% of rats rapidly transition to high levels of drug-taking. This study assessed the SUD-
36 like phenotypes of male and female Sprague Dawley rats self-administering MDPV (0.032
37 mg/kg/infusion) or cocaine (0.32 mg/kg/infusion) by comparing level of drug intake,
38 responding during periods of signaled drug unavailability, and sensitivity to footshock
39 punishment to test the hypotheses that: (1) under short-access conditions, rats that self-
40 administer MDPV will exhibit a more robust SUD-like phenotype than rats that self-
41 administered cocaine; (2) female rats will have a more severe phenotype than male rats;
42 and (3) compared to short-access, long- and intermittent-access to MDPV or cocaine self-
43 administration will result in a more robust SUD-like phenotype. After short-access, rats
44 that self-administered MDPV exhibited a more severe phenotype than rats that self-
45 administered cocaine. Though long- and intermittent-access to cocaine and MDPV self-
46 administration altered drug-taking patterns, manipulating access conditions did not
47 systematically alter their SUD-like phenotype. Evidence from behavioral and quantitative
48 autoradiography studies suggest that these differences are unlikely due to changes in
49 expression levels of dopamine transporter, dopamine D_2 or D_3 receptors, or 5-HT$_{1B}$, 5-HT
50 $_{2A}$, or 5-HT$_{2C}$ receptors, though these possibilities cannot be ruled out. These results show
51 that the phenotype exhibited by rats self-administering MDPV differs from that observed
52 for rats self-administering cocaine, and suggests that individuals that use MDPV and/or
53 related cathinones may be at greater risk for developing a SUD, and that short-access
54 MDPV self-administration may provide a useful method to understand the factors that
55 mediate the transition to problematic or disordered substance use in humans.

Introduction

The use of psychoactive substances has been commonplace for millennia, yet only 15-30% of individuals that use these drugs develop a substance use disorder (SUD) [1]. The DSM-5 defines SUD by 11 diagnostic criteria from four different modalities [2], resulting in a heterogeneous disorder, the severity of which depends on the number of positive criteria [1]. In recent decades, preclinical SUD research has attempted to model the heterogeneity and multi-symptomatic complexities of SUD in animals by assessing multiple behavioral endpoints thought to be related to the diagnostic criteria for SUD [3-6]. These multifaceted studies aim to provide greater insight into SUD and help close the translational gap in the development of effective treatments.

The 3-criteria model, developed by Deroche-Gamonet and colleagues, was one of the first attempts to quantitatively assess the SUD-like phenotypes of rats self-administering cocaine based upon (1) the number of responses made during signaled periods of drug unavailability ("drug-seeking"), (2) breakpoints under progressive ratio schedule of reinforcement ("motivation to use drug"), and (3) resistance to footshock punishment ("continued use despite adverse consequences") [3]. Since then, this model has been adapted and used in numerous studies to identify and assess differences between rats with robust and mild SUD-like phenotypes [4-9]. Initial studies used short-access procedures, where drug was typically available during 1-2 hour sessions; however, subsequent studies suggest that providing longer periods of access to drug (e.g., 6 hrs; long-access) [10] can enhance resistance to footshock punishment [11-13], and increases in cue- and drug-induced reinstatement of extinguished responding [14-16]. More recently, an intermittent-access procedure (5-min of drug availability provided every 30-min over a 6-hour session) was developed to establish rapid, binge-like patterns of cocaine intake [17,18] and has been shown to increase the reinforcing effectiveness of cocaine, and enhance cue- and drug-induced reinstatement of responding [6,19,20]. Thus, while it is possible to observe individual differences in SUD-related behaviors in rats self-administering cocaine (or other drugs) under short-access conditions, mounting evidence suggests when rats are provided long- or intermittent-access to cocaine they develop more severe SUD-like phenotypes (for review, see [21]).

3,4-methylenedioxypyrovalerone (MDPV) is a synthetic cathinone that functions as a cocaine-like monoamine uptake inhibitor, but unlike cocaine which is roughly equipotent at the dopamine, norepinephrine, and serotonin transporters (DAT, NET, and SERT, respectively) MDPV is ~800-fold selective for DAT and NET over SERT [22-24]. We have previously reported that 30-40% of male and female rats that are allowed to self-administer MDPV during daily 90-min sessions rapidly develop a high-responder phenotype, characterized by levels of MDPV intake ~2-5 times greater than low-responders across a range of doses, greater breakpoints under progressive ratio schedules of reinforcement, and higher rates of responding during periods of signaled drug unavailability [25-29]. Though consistent with MDPV high-responder rats engaging in SUD-like behaviors, it is unclear if the phenotype observed in these rats would extend to other core SUD-related behaviors, such as resistance to punishment by footshock,

98   (i.e., "continued use despite adverse consequences"), or if the "severity" of the SUD-like
99   phenotype is sensitive to access condition manipulations, as has been reported for
100   cocaine. Thus, the primary goals of the current study were to: (1) directly compare the
101   SUD-like phenotype of rats self-administering MDPV to those of rats self-administering
102   cocaine under short-access conditions; (2) determine whether long- and intermittent-
103   access to MDPV and cocaine self-administration differentially impacted the SUD-like
104   phenotypes relative to rats maintained on short-access MDPV and cocaine self-
105   administration; and (3) assess whether any of these effects differed as a function of sex.

106   In addition, because stimulant use is known to dysregulate dopaminergic and
107   serotonergic systems, the current studies also assessed how various drug histories (e.g.,
108   MDPV vs. cocaine, short-, long- and intermittent-access), and ultimately the severity of
109   SUD-like phenotype impacted the expression of key transporters and receptors within the
110   dopamine and serotonin systems. Specifically, we used quantitative autoradiography
111   within the caudate putamen and nucleus accumbens to investigate transporters and
112   receptors that have been shown to be increased (i.e., DAT and dopamine D_3 receptors
113   [30-43]; SERT and 5-HT$_{2A}$ receptors [44-47]) or decreased (i.e., dopamine D_2 receptors
114   [48-56], but see [57,58]; 5-HT$_{1B}$ and 5-HT$_{2C}$ receptors [59-61]) following periods of
115   stimulant use. Thus, the overarching goals of these studies were to determine the degree
116   to which the severity of a SUD-like phenotype was influenced by the self-administered
117   drug (MDPV and cocaine), access conditions associated with SUD-like behaviors (short-
118   , long-, and intermittent-access), and the sex of the subject (female and male), and
119   whether key neurobiological changes in dopamine and serotonin systems differed as a
120   function of their SUD-like phenotype severity. Ultimately by identifying conditions that
121   facilitate the development of robust SUD-like phenotypes, these studies will inform future
122   work aimed at identifying medications capable of normalizing aberrant drug-taking
123   behavior in the hopes of developing novel and effective treatments for stimulant and other
124   substance use disorders.

125

Materials and Methods

126

127   *Subjects*

128   Female and male Sprague Dawley rats (weighing 200-225g and 275-300g, respectively,
129   upon arrival) were obtained from Envigo (Indianapolis, IN, USA) and singly housed in a
130   temperature- and humidity-controlled environment under a 14/10-hour light cycle (lights
131   on at 06:00) with *ad libitum* access to Purina chow and water. All experiments were
132   conducted in accordance with the Institutional Animal Care and Use Committee of the
133   University of Texas Health Science Center at San Antonio, and the Guide for Care and
134   Use of Laboratory Animals [62].

135

136   *Surgery*

137 Rats were anesthetized using 2% isoflurane and surgically prepared with a chronic
138 indwelling catheter in the left femoral vein, which was attached to a vascular access
139 button secured in the mid-scapular region, as previously described [26-28,63,64].
140 Penicillin G (60,000 U/rat) or Excede (20 mg/kg) was administered subcutaneously
141 following surgery, and catheters were flushed daily with 0.5 ml heparinized saline (100
142 U/ml) to maintain catheter patency.

143

144 ***Self-Administration***

145 *Apparatus*

146 Intravenous drug self-administration was conducted in standard operant chambers (Med
147 Associates Inc, St. Albans, VT) within light- and sound-attenuated cubicles. A white house
148 light was located on the top of the wall opposite the two levers. Above each lever was a
149 set of red, yellow, and green LEDs. The grid floor was connected to a scrambled shock
150 system (Env-414, Aversive stimulator/scrambler; Med Associates Inc, St. Albans, VT)
151 used to deliver footshocks. A variable-speed syringe driver was used to deliver infusions
152 through Tygon tubing that was connected to a fluid swivel and spring tether held by a
153 counterbalanced arm. The active lever (counterbalanced across rats) was signaled by
154 illumination of the yellow LED above the lever; completion of the response requirement
155 (fixed ratio [FR] 1 or 5) resulted in delivery of the drug infusion and initiation of the 5-sec
156 post-infusion timeout (TO), signaled by illumination of the houselight and all three LEDs
157 above the active lever.

158 *Experimental overview*

159 As shown in Figure 1, rats initially underwent a self-administration training period where
160 they were allowed to self-administer cocaine (0.32 mg/kg/infusion) or MDPV (0.032
161 mg/kg/infusion) for 24 sessions, with the first 14 sessions being under a fixed ratio (FR)
162 1:TO 5-sec schedule of reinforcement, and the remaining 10 being a FR5:TO 5-sec
163 schedule. The doses were selected because of their position on the descending limb of
164 the FR dose response curve and due to the 10-fold potency difference [22,28,29,65,66].
165 Then rats underwent a two-part phenotyping procedure where four endpoints were
166 measured to generate a phenotype score. After this, rats were assigned to self-administer
167 under short (FR5:TO 5-sec; 60-min session), long (FR5:TO 5-sec; 6-hr session), or
168 intermittent access (FR5:TO 1.5-sec; 5 min of drug availability followed by 25-min of drug
169 unavailability in a 6-hr session) for 3 weeks before going through the phenotyping period
170 a second time to test the effects of access condition. Finally, rats underwent a 3-week
171 drug-free period before doing a cue reactivity test where drug was signaled to be
172 available, but only saline was delivered upon completion of the FR5:TO 5-sec schedule
173 of reinforcement. Dopamine D_3 receptor sensitivity was also measured using
174 pramipexole-induced yawning (see Supplemental Methods) before self-administration
175 began and at the end of the study, prior to euthanasia for quantitative autoradiography
176 studies (see Supplemental Methods).

177

Phenotyping procedure

179 To test the effects of access condition on an overall phenotype score, rats underwent a
180 phenotyping procedure before and after the three-week access condition manipulation.
181 The overall phenotype score was a composite score from four endpoints: (1) number of
182 infusions, (2) pre-session responses, (3) intercomponent interval responses, and (4)
183 punishment score. The first three endpoints were collected during the multiple component
184 self-administration and the punishment score was generated during the footshock
185 punishment procedure (see below and Figure 1 for more details).

186 To generate a phenotype score, all rats, regardless of sex or drug, were rank ordered for
187 each endpoint (i.e., pre-session responses, infusions, intercomponent interval responses,
188 and punishment score) and divided by quartiles. Rats in the bottom quartile received a
189 score of 0, rats middle two quartiles received a score of 1, and rats in the top quartile
190 received a score of 2; statistical outliers received scores of 3 [(1.5 x interquartile range) +
191 3rd quartile cutoff] or 4 [(3 x interquartile range) + 3rd quartile cutoff]. Individual phenotype
192 scores represent the mean of the four endpoint scores, with the lowest possible score
193 being 0, and highest possible score being 4. Rats with an overall phenotype score of less
194 than 1 were classified as "low score", rats with a score ≥1 but <2 were classified as "mid
195 score", and rats with a score of ≥2 were classified as having a "high score".

196

Phenotyping procedure: Multiple component self-administration

198 To assess responding during periods of signaled drug availability (and unavailability), all
199 rats were transitioned to a multiple-component schedule of reinforcement that began with
200 a 5-min pre-session TO, followed by three 20-min periods of drug availability, each
201 followed by 5-min intercomponent TOs. During the pre-session and intercomponent TOs,
202 drug was signaled to be unavailable by extinguishing all visual stimuli, and responses
203 were recorded but had no scheduled consequence. A yellow LED above the active lever
204 was used to signal drug availability. The total number of infusions earned, responses
205 made during the presession TO, and responses during the three intercomponent TOs
206 (i.e., periods of drug unavailability) served as endpoints for the phenotype score.

207

Phenotyping procedure: Footshock punishment

209 To assess sensitivity to footshock punished responding, all rats responded under an
210 FR5:TO 5-sec schedule for two 60-min "baseline" sessions before initiating footshock
211 testing. Punishment sessions were identical to "baseline" sessions with the exception that
212 beginning with the 4th infusion, a 0.5-sec, unsignaled footshock was delivered coincident
213 with 1 out of every 2 infusions (i.e., ~50% of infusions were paired with a footshock). The
214 initial footshock intensity was 0.1 mA, and this increased by 0.2 mA across consecutive
215 sessions (0.1, 0.3, 0.5, 0.7mA) until the number of infusions earned was ≤20% of

216 baseline, or a maximum intensity of 0.7mA (Figure 1). Punishment sessions were
217 followed by at least two "baseline" sessions. A punishment score was calculated by
218 multiplying the current that reduced responding by 50% (IC_{50}) with the total infusions
219 earned, and this was fourth endpoint of the phenotype score.

220

Observation of behavioral response to non-contingent footshock

222 To determine if sensitivity to footshock differed across groups, rats were allowed to
223 habituate to a self-administration chamber for 2-5 minutes before receiving a series of
224 non-contingent footshocks. Behavioral responses were scored by a trained observer
225 using the following criteria: 0=no reaction; 1=looks around or passive movement (no
226 startle response); 2=runs around or walks backward rapidly; 3=jump; 4=vocalize.
227 Footshock functions were generated in triplicate, twice in ascending order, and once in
228 descending order. Scores were averaged across the three replicates.

229

Drugs

231 Racemic MDPV was synthesized and supplied by Kenner Rice and cocaine hydrochloride
232 was provided by National Institute on Drug Abuse Drug Supply Program. Both self-
233 administered drugs were dissolved in sterile, physiological saline and delivered
234 intravenously at a volume of 0.1 ml/kg and a dose of 0.032 mg/kg/infusion for MDPV and
235 0.32 mg/kg/infusion for cocaine.

236

Statistical analyses

238 A one-factor (score) ANOVA was used for rate and level of acquisition with Tukey's post-
239 hoc analyses (Table 1). Raw data for infusions, intercomponent TO responses,
240 punishment score, and rate of responding were log-transformed before analyzing using
241 two-factor or three-factor ANVOAs. Two-factor or three-factor ANOVAs were performed
242 on the raw values for pre-session responses, cue reactivity responses, phenotype score,
243 and change in phenotype score. Two-factor (drug x sex) ANOVAs were conducted for the
244 dataset in Figure 1, and three-factor (drug x sex x access condition or drug x sex x
245 phenotype score) ANOVAs were performed on datasets in Figures 2-4. Tukey's post-hoc
246 analyses were performed when there was a significant main effect of access condition or
247 phenotype score. Change in phenotype score (Figure 3F) was analyzed by calculating
248 the mean change in phenotype score (phenotype score 2 – phenotype score 1) and
249 comparing 95% confidence intervals, which were corrected for multiple comparisons.
250 Escalation (Table 2) was initially calculated in individual subjects (mean of the last three
251 sessions – mean of first 3 sessions of the access condition manipulation). The mean and
252 95% confidence intervals were used to compare whether there was significant escalation
253 (confidence intervals did not overlap with 0). The behavioral response to noncontingent
254 shock was analyzed using a two-factor (shock intensity x group) ANOVA, where group

255   was phenotype score, self-administered drug, or sex. Sidak's multiple comparisons post-
256   hoc analyses were performed when there was a main effect of group. Data from a subset
257   of rats (n=8; n=2 per sex/drug) were excluded from the cue reactivity test due to
258   procedural error.

259

260   **Results**

261   ***Acquisition of cocaine and MDPV self-administration***

262   Nearly all rats met acquisition criteria (i.e., ≥ 20 infusions and ≥ 80% of responses on the
263   active lever) within the 14-session acquisition period. Although rats self-administering
264   MDPV acquired earlier (~4.5 sessions) than rats self-administering cocaine (~7.5
265   sessions), the rate of MDPV and cocaine acquisition did not vary by sex (Table 1). The
266   level of acquisition (i.e., mean infusions earned during sessions 12-14) did not differ as a
267   function of either sex or drug (Table 1). Retrospective analyses revealed that rats with
268   higher phenotype scores at the initial phenotyping period acquired more quickly and
269   earned more infusions than rats with lower phenotype scores (Table 1).

270

271   ***Phenotype 1***

272   After the initial phenotyping period (i.e., ~5 weeks of short access self-administration), a
273   two-factor (drug x sex) ANOVA revealed main effects of both sex ($F_{[1, 114]}$ = 5.91;
274   p=0.017) and drug ($F_{[1, 114]}$ = 26.09; p<0.0001), where females had higher scores than
275   males, and rats that self-administered MDPV had higher phenotype scores than rats that
276   self-administered cocaine (Figure 1E). This trend was generally true for each of the
277   individual phenotype endpoints where analysis of the mean number of infusions found
278   main effects of sex ($F_{[1, 114]}$ = 7.38; P=0.0076), with females earning more infusions
279   than males, and drug ($F_{[1, 114]}$ = 9.28; P=0.0029), where more infusions of MDPV were
280   self-administered than cocaine (Figure 1A). Similarly, analysis of pre-session TO
281   responses revealed females made more responses than males ($F_{[1, 114]}$ = 10.40;
282   P=0.0016) and rats self-administering MDPV made more responses than rats self-
283   administering cocaine ($F_{[1, 114]}$ = 36.56; P<0.0001) (Figure 1B). Analysis of
284   intercomponent TO responses found that females responded more than males ($F_{[1, 114]}$
285   = 7.47; P=0.0073) and rats self-administering MDPV responded more than rats self-
286   administering cocaine ($F_{[1, 114]}$ = 42.63; P<0.0001) (Figure 1C). In contrast, though rats
287   self-administering MDPV had higher punishment scores than rats self-administering
288   cocaine ($F_{[1, 114]}$ = 10.28; P=0.0017), there was no main effect of sex ($F_{[1, 114]}$ = 0.22;
289   P=0.6436) (Figure 1D). Importantly, the behavioral response to noncontingent footshock
290   did not differ as a function of SUD-like phenotype score or self-administration drug
291   (phenotype score: $F_{[2, 115]}$ = 0.17); P=0.8453; drug: $F_{[1, 116]}$ = 0.17; P=0.6795)
292   (Supplemental Figure 1), however, females were more sensitive than males ($F_{[1, 116]}$ =
293   11.27; P=0.0011) (Supplemental Figure 2). There were no sex x drug interactions for any
294   behavioral endpoints or the overall phenotype score.

295

Access conditions

297   The mean number of infusions and rate of responding over the 21-day access condition
298   manipulation are shown in Figure 2. 3-factor ANOVA (access condition x drug x sex)
299   revealed significant main effects of access for both number of infusions earned ($F_{[2, 106]}$
300   = 108.40; P<0.0001), where long > intermittent > short, and rate of responding ($F_{[2, 106]}$
301   = 41.14; P<0.0001), where intermittent > long = short. Consistent with the first
302   phenotyping period, rats that self-administered MDPV earned more infusions ($F_{[1, 106]}$
303   = 13.70; P=0.0003) and responded at a faster rate [$F_{(1, 106)}$ = 13.76; P=0.0003] than
304   rats that self-administered cocaine. Similarly, females earned more infusions ($F_{[1, 106]}$
305   = 10.23; P=0.0018) and responded at a faster rate ($F_{[1, 106]}$ = 10.22; P=0.0018) than
306   males. Rats that self-administered cocaine, but not MDPV, under long-access conditions
307   showed a significant escalation in drug intake (Table 2).

308

Phenotype 2

310   Redeterminations of the SUD-like phenotype score after manipulating access condition
311   are shown in Figure 3. There were no main effects of access condition on any of the
312   behavioral endpoints nor the overall phenotype score. However, as was observed during
313   the initial phenotyping period, there were main effects of sex and drug on multiple
314   endpoints, as well as the overall phenotype score. Rats that self-administered MDPV
315   earned more infusions ($F_{[1, 106]}$ = 20.58; P<0.0001), made more pre-session TO
316   responses ($F_{[1, 106]}$ = 15.14; P=0.0002), made more intercomponent TO responses (F
317     $_{[1, 106]}$ = 19.22; P<0.0001), and had an overall higher phenotype score than rats self-
318   administering cocaine ($F_{[1, 106]}$ = 11.73; P=0.0009). Females earned more infusions (F
319     $_{[1, 106]}$ = 8.71; P=0.0039), made more pre-session TO responses ($F_{[1, 106]}$ = 5.24;
320   P=0.0240), and had higher phenotype scores ($F_{[1, 106]}$ = 5.36; P=0.0226) compared to
321   males. Though there was no effect of access condition on the overall phenotype score,
322   analysis of the change in phenotype score (Figure 3F) revealed that long-access to
323   cocaine resulted in a significant increase in phenotype score for female rats (mean: 0.44;
324   95% CI: 0.07-0.82). After self-administration concluded, sensitivity to noncontingent
325   footshock was measured. There was no difference between rats with low, mid, and high
326   phenotype scores or between rats self-administering MDPV or cocaine; however, female
327   rats were more sensitive than male rats (Figure S1).

328

Cue reactivity

330   The number of responses made during the cue reactivity test (i.e., for drug-paired stimuli
331   and a saline infusion) is shown in Figure 4. There were significant main effects of access
332   condition ($F_{[2, 106]}$ = 5.21; P=0.0069), drug ($F_{[1, 106]}$ = 5.05; P=0.0268), and sex (F [1,
333   106] = 5.74; P=0.0183), where rats that previously self-administered MDPV made more
334   responses than rats that had self-administered cocaine, and females made more

335 responses than males (Figure 4A). Post-hoc analyses revealed that rats with a history of
336 intermittent-access self-administration also made more responses than rats that self-
337 administered under short- (P=0.0126) or long-access (P=0.0290) conditions; responding
338 by short- and long-access rats did not differ (P=0.9538). There were no significant
339 interactions (P≥0.2526). When cue reactivity responses were analyzed by phenotype
340 score, there were no significant main effects (P≥0.0774) or interactions (P≥0.2424)
341 (Figure 4B).

342

343 ***Measures of receptor sensitivity or availability***

344 Behavioral responses (yawning) to non-contingent administration of pramipexole, a
345 dopamine D_3/D_2 receptor agonist, and lorcaserin, a $5-HT_{2C}$ receptor agonist, were
346 evaluated in a subset of rats (n=43), both prior to initiating self-administration and again
347 after the cue-reactivity tests. Pramipexole dose-dependently induced yawning in male
348 and female rats, although females yawned about half as much as males (Figure S2).
349 There were no effects of phenotype score at either time point; however, there was a main
350 effect of time on the composite yawning score (minimally effect dose x peak number of
351 yawns) in both female (F [1, 63] = 14.70; P=0.0003) and male rats (F (2, 59) = 19.46;
352 P<0.0001) where rats had a higher composite yawning score after self-administration
353 compared to before self-administration began (Figure S2). Lorcaserin did not reliably
354 induce yawning in most rats (data not shown).

355

356 Quantitative autoradiography studies were conducted on brain tissue collected from a
357 subset of rats (n=60) after the cue reactivity tests. Expression levels of the dopamine
358 transporter and dopamine D_2, dopamine D_3, $5-HT_{1B}$, $5-HT_{2A}$, or $5-HT_{2C}$ receptors did not
359 vary as a function of phenotype score or sex in the nucleus accumbens or caudate
360 putamen (Figures S3, S4, S5). However, there was an effect of access condition
361 (intermittent > short) and drug (MDPV > cocaine) for increased $5-HT_{1B}$ and $5-HT_{2C}$
362 receptor expression, respectively (Figures S4, S5, S6).

363

364 **Discussion**

365 Similar to the heterogeneous manifestation of SUD in people, rats can develop different
366 levels of SUD-related behaviors. Studying rats with more extreme phenotypes may
367 provide a more translational framework to understand factors that underlie the transition
368 from regular to disordered patterns of substance use. Though a relatively small subset of
369 rats (17-22%) develop the most severe SUD-like phenotype when they are allowed to
370 self-administer cocaine [3,8], a much larger proportion of rats (~30-40%) engage in
371 aberrantly high levels of drug-taking when MDPV is available for self-administration [25-
372 29]. Thus, the primary goals of the current studies were to directly compare the SUD-like
373 phenotype in male and female rats self-administering MDPV or cocaine, and to determine
374 how manipulating access condition (short-, long-, and intermittent-access) impacted

375 these SUD-like phenotypes. The first central finding was that rats that self-administer
376 MDPV have a more robust SUD-like phenotype than rats that self-administer cocaine
377 after an initial period of short-access self-administration (Figure 1). Second, female rats
378 exhibit a more robust phenotype than male rats (Figures 1, 3). Third, providing rats with
379 long- or intermittent-access to MDPV or cocaine self-administration did not alter the
380 severity of their SUD-like phenotype, except for female rats self-administering cocaine
381 under long-access conditions, which had increased scores during the second
382 phenotyping period (Figure 3). Finally, evidence from behavioral and quantitative
383 autoradiography studies suggests that these differences may not be due to shifts in
384 expression level of DAT, dopamine D_2 or D_3 receptors, or $5-HT_{1B}$, $5-HT_{2A}$, or $5-HT_{2C}$
385 receptors (but see SI discussion).

386 Consistent with previous studies reporting unusually high levels of drug-taking in male
387 and female rats that self-administer MDPV, rats that self-administered MDPV had a more
388 severe SUD-like phenotype score than rats that self-administered cocaine, regardless of
389 access condition or duration of self-administration. This was primarily due to the increase
390 in infusions earned, responses made during the pre-session time out, and responses
391 made when drug was signaled to be unavailable, replicating and extending our previous
392 studies with high-responder rats [25-29]. Given that sensitivity to punishment frequently
393 contributes to severe SUD-like phenotype [3,4,6-8] and considering some rats that self-
394 administered MDPV had very high punishment scores, it was somewhat unexpected that
395 rats that self-administered cocaine or MDPV did not differ with regard to the punishment
396 endpoint. This was especially surprising given that the punishment score incorporated
397 both footshock sensitivity (IC_{50}) and total current received, and some rats self-
398 administering MDPV earned several dozen more infusions than rats self-administering
399 cocaine. Perhaps the footshock schedule (unsignaled, and unable to be avoided without
400 suppressing all responding) masked any differences between the groups and another
401 procedure (e.g., signaled footshock) would tease apart differences between rats self-
402 administering MDPV or cocaine. Alternatively, the phenotype that leads to sensitivity to
403 footshock punishment may be related less to the other behavioral endpoints [67].

404 Women initiate drug use later than men, but transition from initial substance use to
405 treatment-seeking in a shorter time period and use similar amounts of cocaine as men
406 [68-71], suggesting women may develop a SUD more rapidly and/or with greater severity
407 compared to men. Even though females and males acquired responding for MDPV and
408 cocaine at similar rates and to similar levels, females self-administering either MDPV or
409 cocaine exhibited more severe SUD-like phenotypes than males during both phenotyping
410 periods. Female rats were more sensitive to noncontingent footshock, suggesting their
411 punishment score and, by extension, overall phenotype score may have even been
412 underestimated.

413 Decades of work suggest that providing rats long periods of access to cocaine self-
414 administration can result in the development of behaviors thought to more closely
415 resemble SUDs in people (e.g., escalated drug intake, resistance to punishment by
416 footshock) [11-16]. More recently, the intermittent access procedure has been shown to

417 promote rapid, binge-like patterns of cocaine use, and increase the reinforcing
418 effectiveness of cocaine [6,17-20]. Both phenomena were observed in the present study,
419 although the escalation was not statistically significant in rats that self-administered
420 MDPV under long-access conditions. Unexpectedly, we found that some rats self-
421 administered up to 80 infusions of MDPV in a single 5-min period during the intermittent-
422 access procedure. Five of 22 of the rats (23%) that self-administered MDPV earned an
423 average of 17-45 infusions per 5-min period across the entire 21-session access
424 manipulation, which is much higher than the approximate 3-12 cocaine infusions in a 5-
425 min period that we and others have observed when unit-doses ranging from 0.25 to 0.4
426 mg/kg/infusion are available [72-76]. This finding strongly supports the notion that binge-
427 like patterns of dysregulated drug-taking develop in a subset of rats that self-administer
428 MDPV, consistent with what has been reported by humans using MDPV and related
429 synthetic cathinones. Though the access manipulations produced robust behavioral
430 differences, these effects did not carry over into the phenotyping period, suggesting that
431 the changes in patterns of drug-taking induced by long- and/or intermittent-access may
432 not be long-lasting and may be more a function of the schedule of reinforcement than a
433 fundamental change in the "state" of the rat. Though we have previously reported that
434 'high-responder" rats will earn significantly more infusions of MDPV under both FR and
435 progressive ratio schedules of reinforcement, we did not evaluate responding under a
436 progressive ratio schedule of reinforcement or use behavioral economics. Other studies
437 find that rats with a history of long- or intermittent-access find cocaine and other
438 reinforcers more reinforcing than rats with a history of short-access self-administration
439 [14,18,72,75,77-82], but see [83].

440 Though rats with a history of self-administering cocaine under long- compared to short-
441 access conditions have been reported to make more responses during reinstatement or
442 cue reactivity tests [14-16], this effect was not seen in the present study. However, rats
443 that self-administered under intermittent-access did make more responses compared to
444 the other access conditions, consistent with other reports [75,76,84]. We also found that
445 female rats made more responses than male rats during the cue reactivity test, which
446 may be related to the higher rates of relapse and drug craving in women compared to
447 men [85-88]; however, many studies do not report sex differences in reinstatement or cue
448 reactivity tests [14,89,90], but see [91]. These differences could be due to procedural
449 differences (e.g., extinction sessions or a history of punishment).

450 The differences in MDPV and cocaine at the first phenotyping period could have
451 represented a quicker transition to a SUD-like phenotype; however, the fact that the
452 phenotype scores for cocaine and MDPV did not converge after access suggests that
453 there is something fundamentally different about the development of SUD-like
454 phenotypes in response to MDPV and cocaine self-administration. Since we did not
455 observe any consistent effects of access condition, this study cannot rule out the
456 involvement of DAT or any of the receptors quantified here in the presentation of SUD-
457 like phenotype(s). Additionally, because all rats showed a leftward shift in the
458 pramipexole-induced yawning dose-response function (Figure S2), the assay may not

459 have been sensitive enough to detect relatively small differences in the size of the shift.
460 Future studies could use alternative approaches, such as RNA sequencing or genome-
461 wide association studies [92-95] to cast a wider net to identify underlying factors that
462 contribute to the individual differences in SUD-like phenotype.

463 The within-subject design can be powerful to evaluate individual differences, with some
464 caveats. For instance, more sessions were spent self-administering under short-access
465 conditions for all groups than the access condition manipulation (70 short-access vs 21
466 of long- or intermittent-access), which may have attenuated the effects of the access
467 condition. However, we calculated the second phenotype score using data from only the
468 first three sessions following the access condition manipulation and did not find any
469 differences compared to using the average of the entire phenotyping period (data not
470 shown). Additionally, the effects of intermittent-access may have been underestimated
471 since two of the phenotype endpoints (pre-session responses and intercomponent
472 timeout responses) measured responding during signaled periods of unavailability, and
473 rats with intermittent-access had extended periods (5-hours/session) of drug
474 unavailability, which rats in the short- and long-access groups did not experience.
475 However, there was also no effect of intermittent-access on the other two endpoints (i.e.,
476 infusions, punishment score), suggesting an overall lack of effect of intermittent-access.

477 Synthetic cathinones have been reported to produce stimulant and euphoric effects in
478 humans [96], and in the current study, even relatively brief periods of short-access to
479 MDPV self-administration produced high levels of drug-taking and -seeking. Though the
480 SUD-like phenotype established by MDPV was not exacerbated by a history of long- or
481 intermittent-access to MDPV self-administration, it is equally interesting and important to
482 note that even long- or intermittent-access to cocaine was unable to produce an SUD-like
483 phenotype comparable to that established with MDPV. Exploiting the severe phenotype
484 developed in rats self-administering MDPV to investigate the mechanisms that underly
485 the development of the phenotype can provide valuable insight into the transition in
486 people from recreational use to SUD and help identify novel pharmacotherapies for SUD
487 treatment.

488

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Competing Interests

The authors have nothing to disclose.

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Author contributions

MRD and GTC conceived the project and designed the experiments with input from GGG and LCD. MRD, MSAB, MS, VA, and MD performed the behavioral experiments. NMB analyzed and scored the yawning videos and contributed to data analysis. MRD, GGG, MSAB, and MS performed quantitative autoradiography experiments and analysis. MRD analyzed and interpreted the experimental data. GTC, KMS, GGG and LCD supervised the research. KCR contributed reagents. MRD and GTC wrote the manuscript with contributions from all authors. All authors read and approved the final manuscript.

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813

814

815 **Table 1.** Acquisition of Cocaine and MDPV Self-Administration.

816 The mean number of days to reach acquisition criteria (>20 infusions and >80% of
817 responses on the active lever), percent of rats that acquired, and level of acquisition
818 (mean infusions / session) in rats, split by sex, self-administration drug and initial
819 phenotype score.

		Days to Acquisition mean ± SEM	% of rats acquired (acquired n / total n)	Level of acquisition mean ± SEM
Cocaine	Females	7.0 + 0.6	93% (28 / 30)	58.3 + 2.7
	Males	7.7 + 0.6	96% (27 / 30)	49.9 + 2.6
MDPV	Females	4.2 + 0.4 *	100% (30 / 30)	69.1 + 7.1
	Males	4.6 + 0.4 *	100% (30 / 30)	64.9 + 13.4
<1 score	All	7.2 + 0.5	98% (48 / 49)	44.6 + 1.6
	Cocaine	*8.8 + 0.5*	*97% (32 / 33)*	*47.4 + 2.0*
	MDPV	*4.4 + 0.5 **	*100% (16 / 16)*	*38.0 + 2.2*
< 2 score	All	5.1 + 0.4 #	96% (51 / 53)	63.6 + 4.1 #
	Cocaine	*6.5 + 0.7*	*92% (22 / 24)*	*62.3 + 3.0*
	MDPV	*4.6 + 0.5 **	*100% (29 / 29)*	*64.7 + 7.1*
2+ score	All	4.0 + 0.4 #$	100% (16 / 16)	102.7 + 22.8 #
	Cocaine	*4*	*100% (1 / 1)*	*74.3*
	MDPV	*4.0 + 0.4*	*100% (15 / 15)*	*104.6 + 24.3 # $*

820

821 * = significantly different than rats of the same sex or score that self-administered cocaine

822 # = significantly different than <1 rats in the same group

823 $ = significantly different than <2 rats

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826

827 **Table 2.** Escalation During 21-Day Access Condition Manipulation

828 Mean and confidence intervals (CI) around escalation, calculated by mean of infusions
829 earned during sessions 19-21 minus mean of sessions 1-3 in individual subjects. Data
830 shown by self-administration group and phenotype score.

Access condition	Group	n	Escalation Mean (95% CI)
Short	Female cocaine	9	0.6 (-8.5, 9.6)
	Male cocaine	9	0.8 (-2.8, 4.4)
	Female MDPV	10	-11.3 (-50.0, 27.4)
	Male MDPV	9	1.1 (-25.3, 27.5)
Long	Female cocaine	9	**39.8 (21.3, 58.2) ***
	Male cocaine	9	**45.0 (12.7, 77.3) ***
	Female MDPV	9	160.8 (-141.1, 462.7)
	Male MDPV	10	35.7 (-15.4, 86.7)
Intermittent	Female cocaine	12	13.8 (-4.5, 32.0)
	Male cocaine	10	0.3 (-23.3, 23.8)
	Female MDPV	11	17.5 (-86.4, 121.3)
	Male MDPV	11	-6.3 (-42.2, 29.7)
Short	<1	14	0.1 (-5.1, 5.3)
	<2	18	1.2 (-10.6, 13.1)
	2+	5	-22.9 (-121.0, 75.3)
Long	<1	10	**40.2 (4.1, 76.2) ***
	<2	22	61.0 (-43, 165.2)
	2+	5	164.5 (-84.9, 413.9)
Intermittent	<1	25	0.3 (-16.9, 17.5)
	<2	14	0.7 (-31.5, 32.8)
	2	5	54.8 (-219.8, 329.4)
Short		37	-2.5 (-13.3, 8.4)
Long		37	**69.4 (3.7, 135.1) ***
Intermittent		44	6.6 (-18.4, 31.6)

831

832 * = significant escalation (confidence intervals do not overlap with 0)

Figure Legends

Figure 1. Timeline and Initial Phenotype Score Endpoints

Experimental timeline showing the total study duration in days as well as each aspect of the experiment. Violin plots representing the mean number of infusions (A), pre-session responses (B), intercomponent interval responses (C), punishment score (D) and SUD-like phenotype score (E) in female (shaded) and male (white) rats self-administering cocaine (red) or MPDV (blue) during the first phenotyping period. Solid lines indicate median and dashed lines indicate quartiles. * =P<0.05, **=P<0.01, ***=P<0.001, ****P=<0.0001 for post-hoc analyses.

Figure 2. Access Condition Manipulations

Violin plots representing the number of infusions (top) and rate of responding (bottom) averaged across the 21-session access condition manipulation. Female (shaded) and male (white) rats self-administering cocaine (red) or MPDV (blue) under short- (left), long- (middle), or intermittent-access (right). Solid lines indicate median and dashed lines indicate quartiles. Main effect of access condition where ****=P<0.0001 compared to short-access; ####=P<0.0001 compared to long-access for post-hoc analyses.

Figure 3. Final Phenotype Score Endpoints

Violin plots representing the mean number of infusions (A), pre-session responses (B), intercomponent interval responses (C), punishment score (D), SUD-like phenotype score (E), and change in phenotype score in female (shaded) and male (white) rats self-administering cocaine (red) or MPDV (blue) during the second phenotyping period. Data are split by access condition. Solid lines indicate median and dashed lines indicate quartiles. *=significant change in phenotype score, where confidence intervals did not overlap with 0. Main effects of drug and sex are not indicated on figures.

Figure 4. Responses During Cue Reactivity Test

Violin plots representing the mean number of responses during the cue reactivity test, split by access condition (top) or phenotype score (bottom) in female (shaded) and male (white) rats self-administering cocaine (red) or MPDV (blue). Solid lines indicate median and dashed lines indicate quartiles. Top: main effect of access condition where *=P<0.05 compared to short-access; #=P<0.05 compared to long-access. Bottom: main effect of phenotype where **=P<0.01 compared to low score; #=P<0.05 compared to mid score.

Figure 1

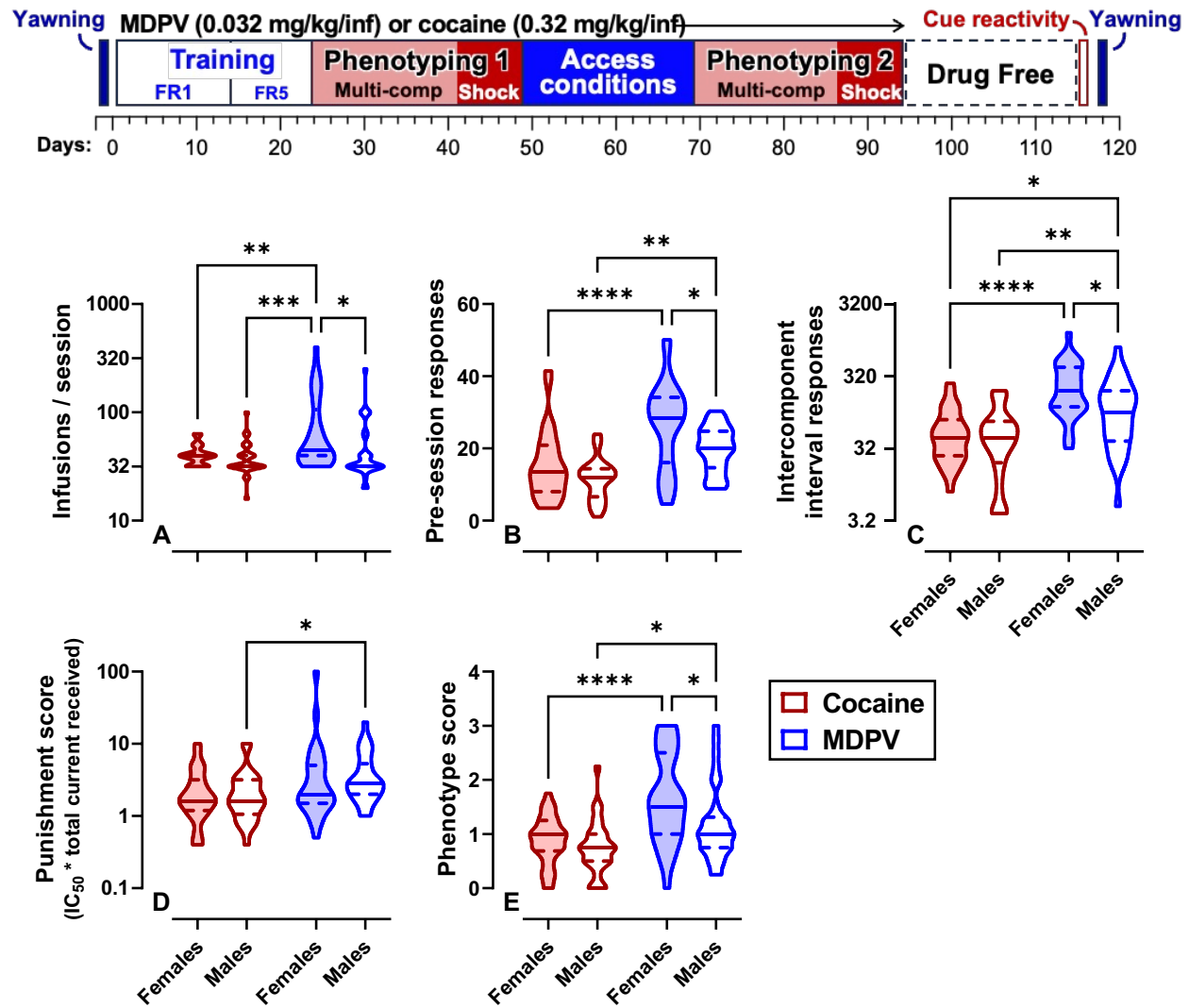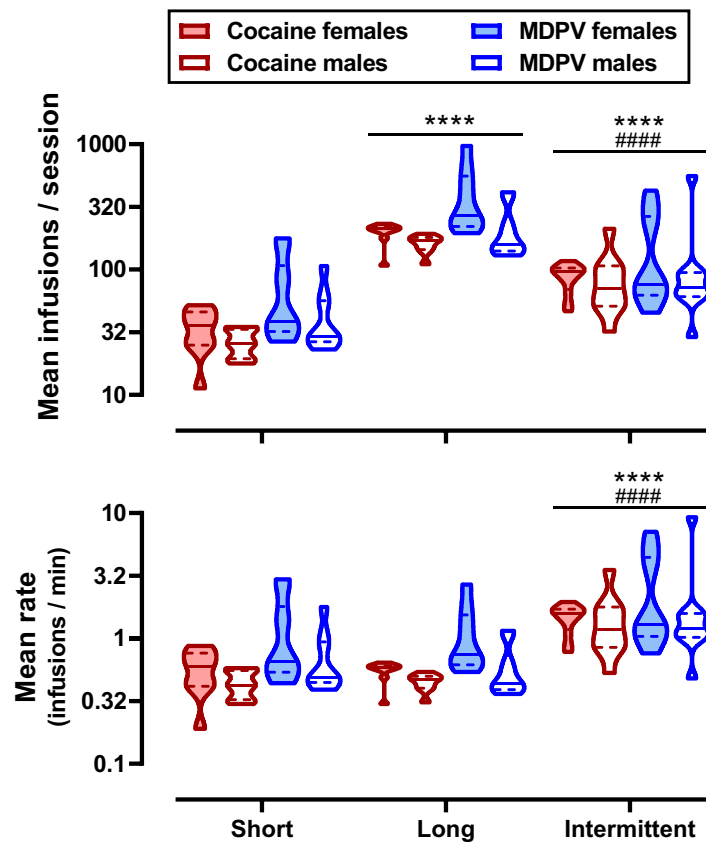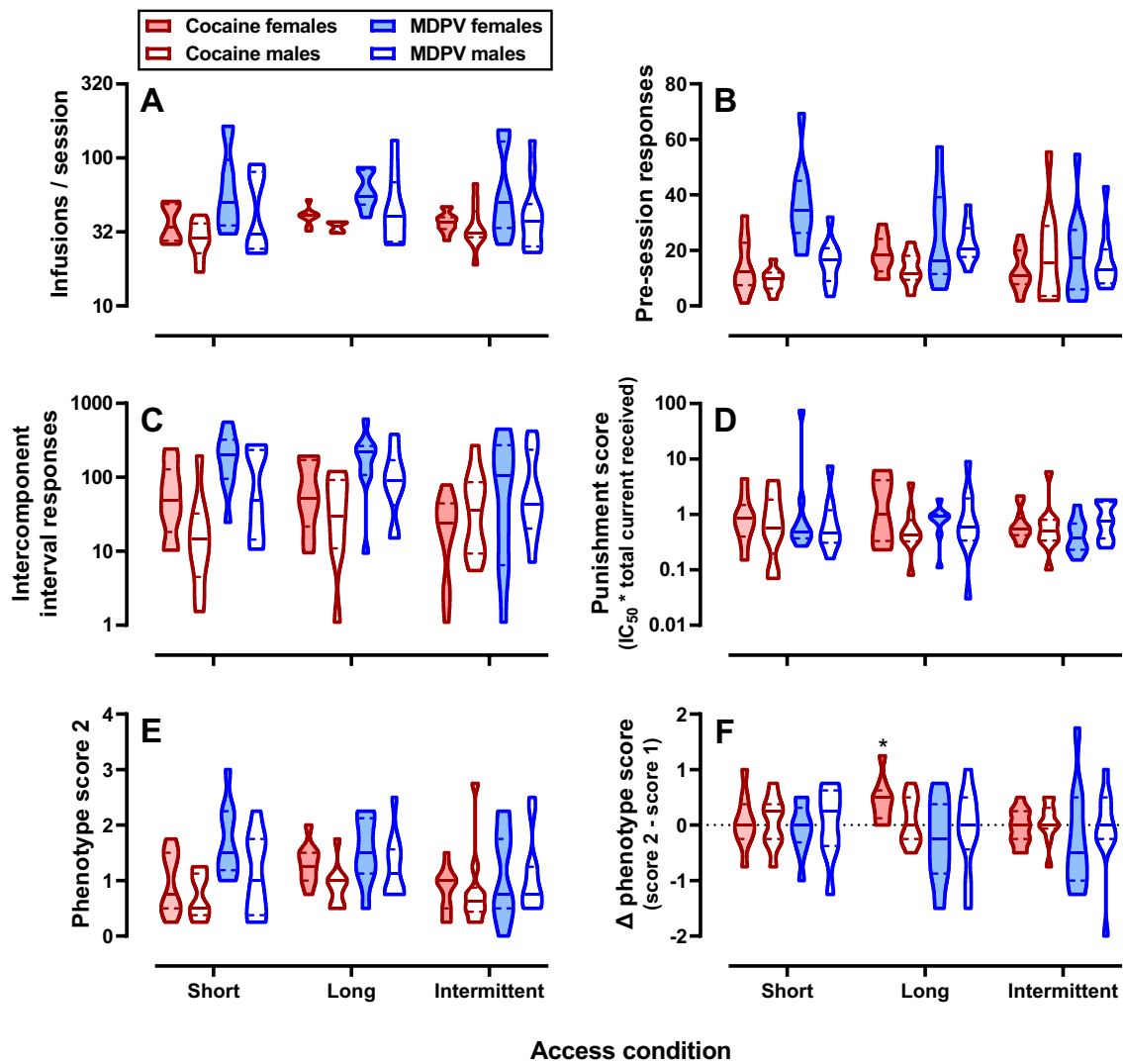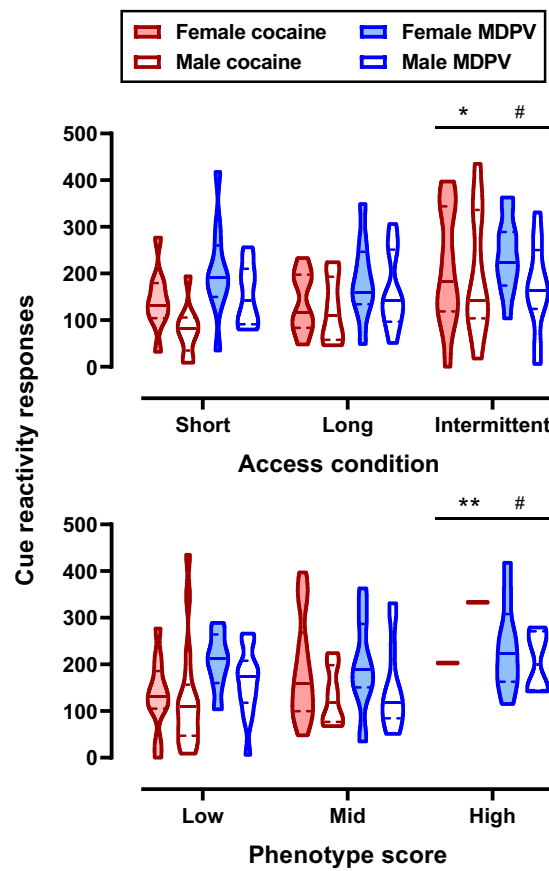Figure 2

Figure 3

Figure 4
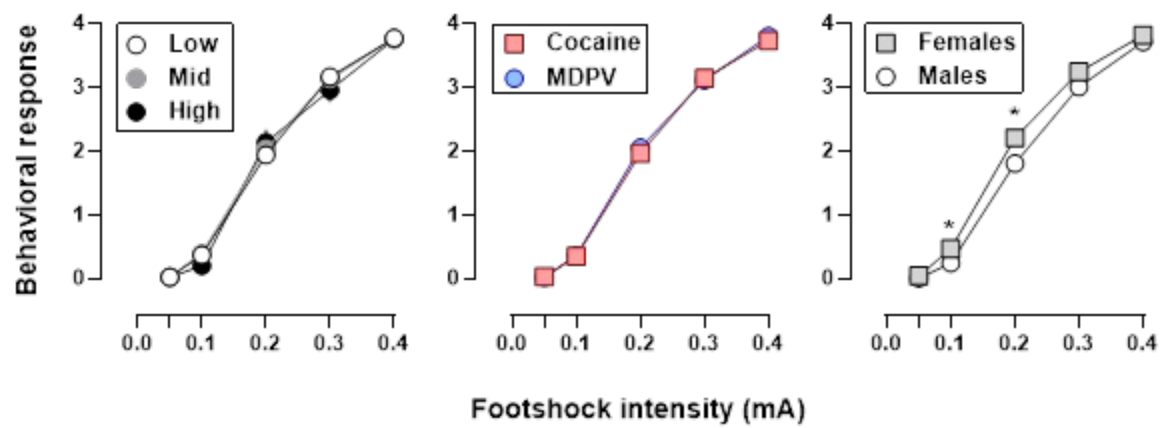
Supplemental Figure 1

Footshock intensity (mA)

Supplemental Figure 2

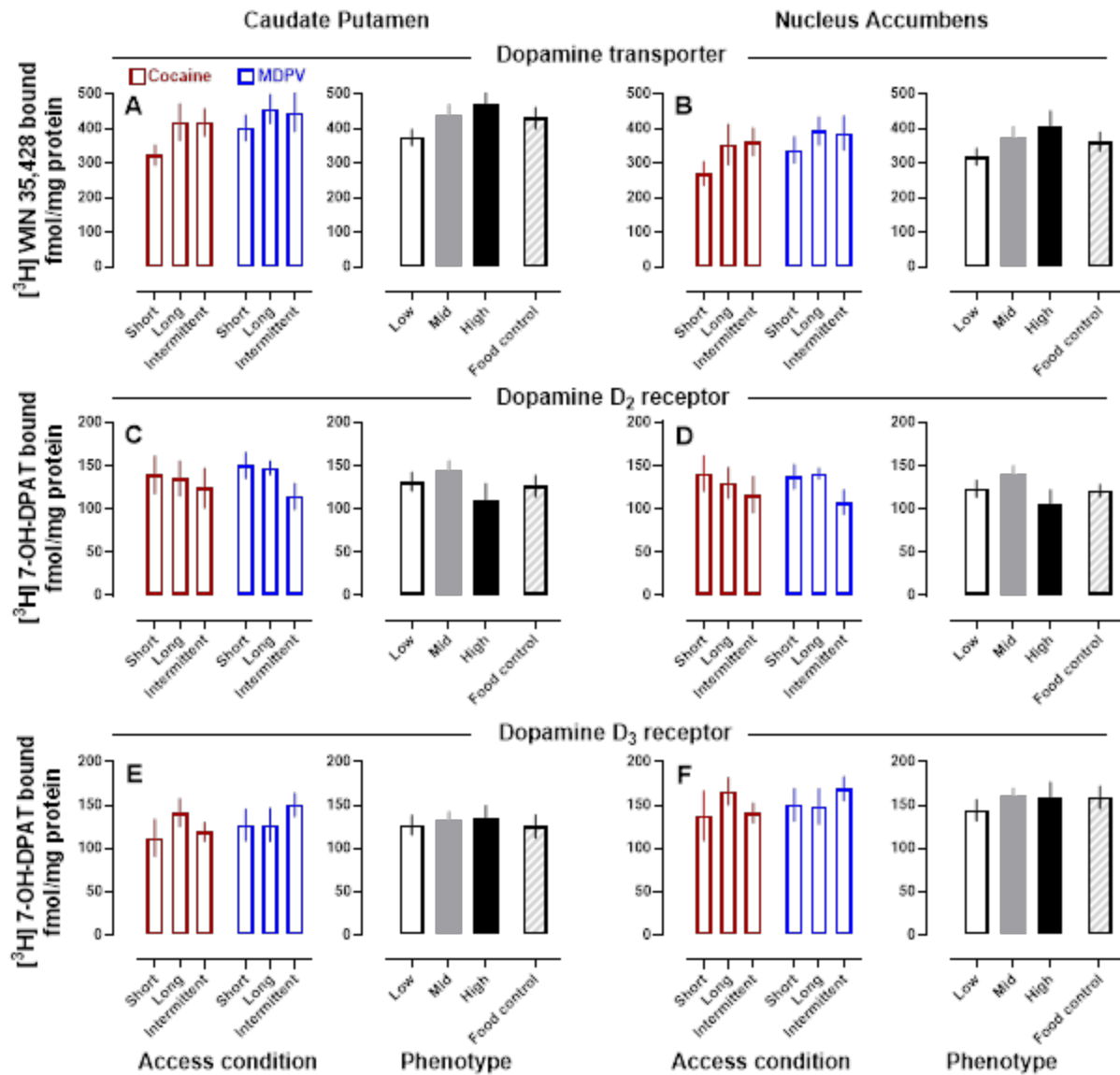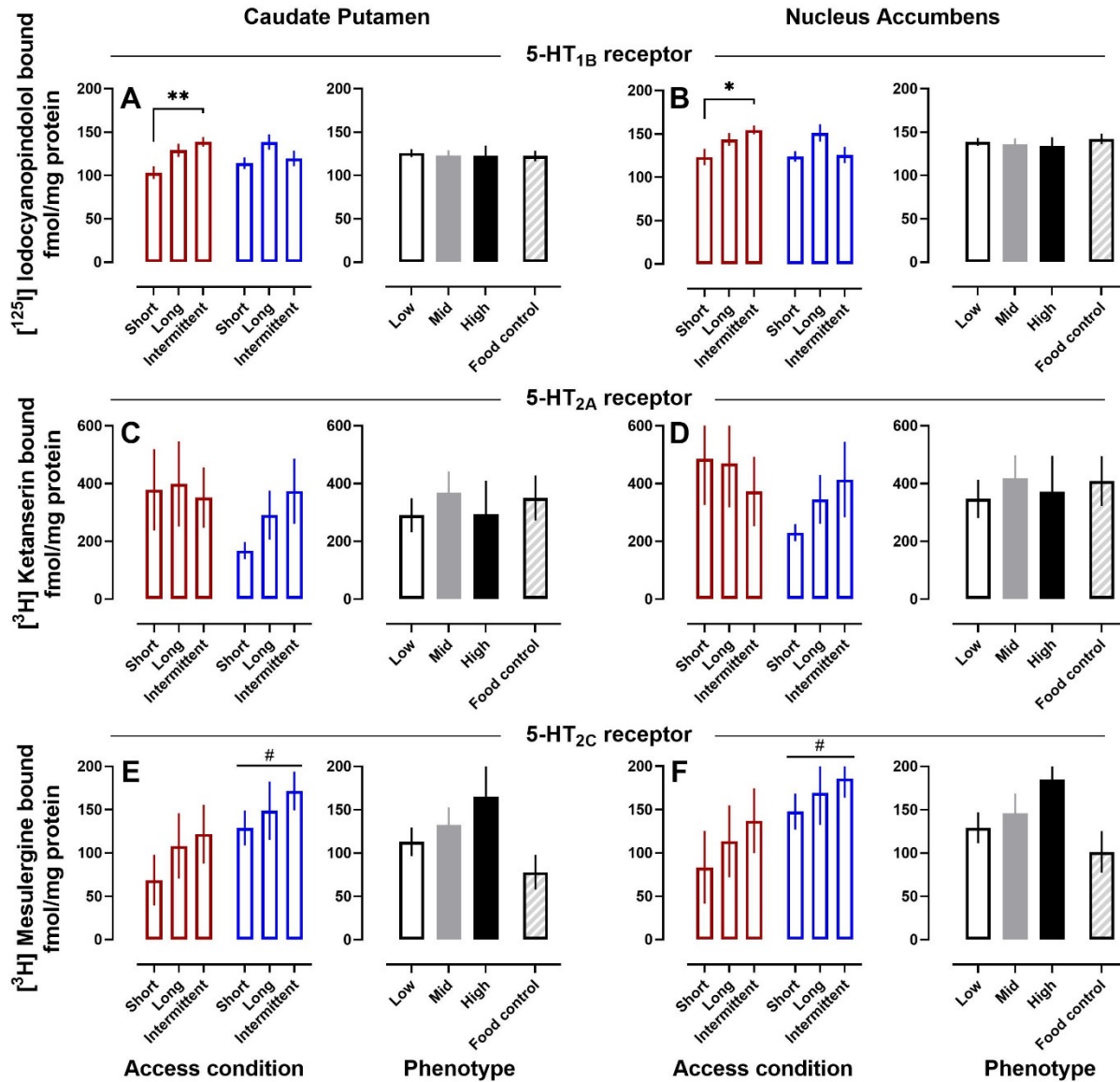# Supplemental Figure 3

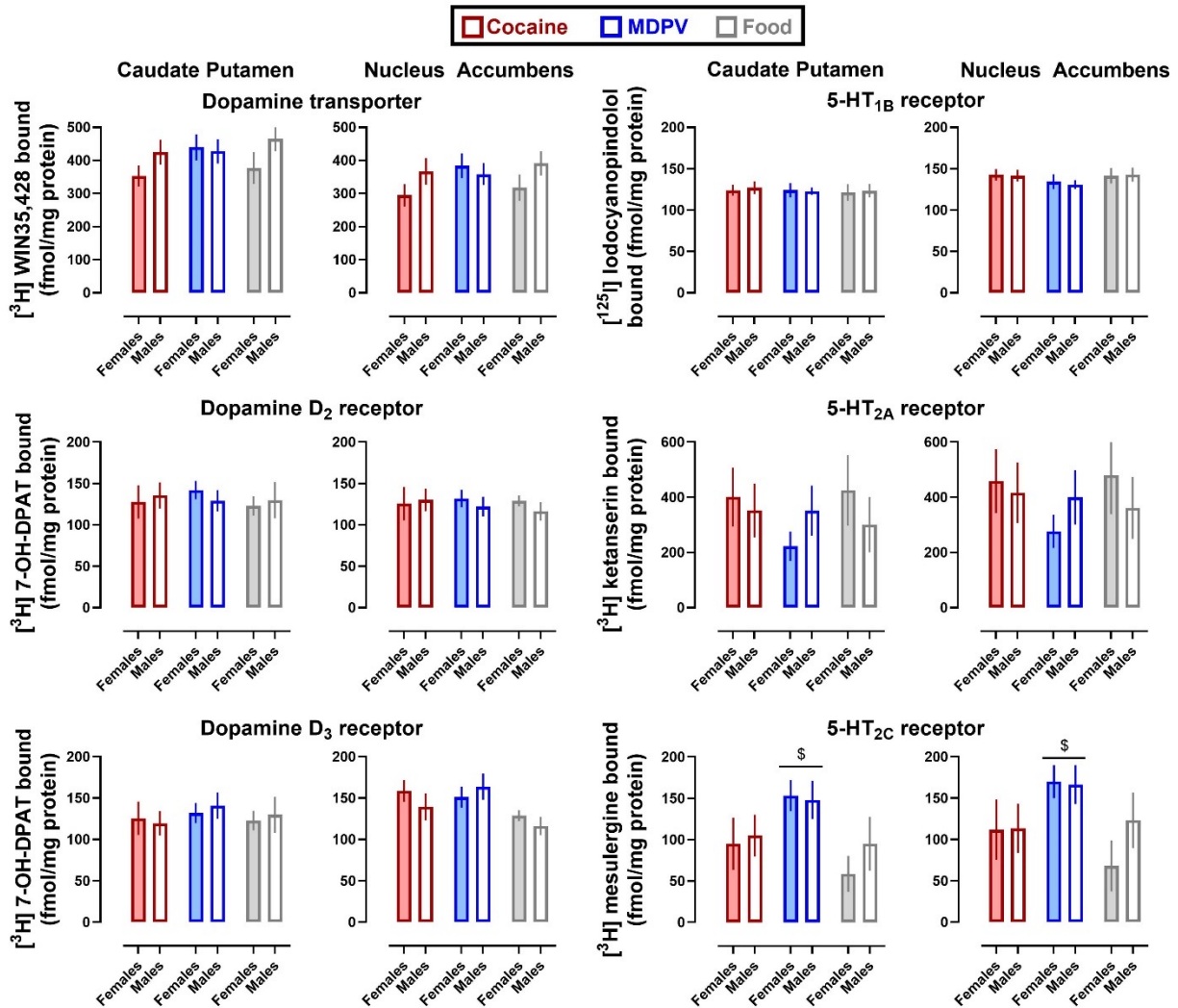# Supplemental Figure 4

Supplemental Figure 5

Supplemental Figure 6