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## **Delay of punishment highlights differential vulnerability to developing addiction-like behavior toward sweet food**

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1           **Title Page**

2           **Title:** Delay of punishment highlights differential vulnerability to developing  
3 addiction-like behavior toward sweet food

4

5           **Running Title:** Delay and resistance to punishment

6

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30 **ABSTRACT**

31 Resistance to punishment is commonly used to measure the difficulty in refraining from  
32 rewarding activities when negative consequences ensue, which is a hallmark of  
33 addictive behavior. We recently developed a progressive shock strength (PSS)  
34 procedure in which individual rats can titrate the amount of punishment that they are  
35 willing to tolerate to obtain food rewards. Here, we investigated the effects of a range  
36 of delays (0-12 sec) on resistance to punishment measured by PSS break points. As  
37 expected from delay discounting principles, we found that delayed shock was less  
38 effective as a punisher, as revealed by higher PSS breakpoints. However, this  
39 discounting effect was not equally distributed in the population of rats, and the  
40 introduction of a delay highlighted the existence of two populations: rats that were  
41 sensitive to immediate punishment were also sensitive to delayed shock, whereas rats  
42 that were resistant to immediate punishment showed strong temporal discounting of  
43 delayed punishment. Importantly, shock-sensitive rats suppressed responding even in  
44 subsequent non-punishment sessions, and they differed from shock-resistant rats in  
45 anxiety-like behavior, but not in sensitivity to pain. These results show that  
46 manipulation of temporal contingencies of punishment in the PSS procedure provides  
47 a valuable tool to identify individuals with a double vulnerability to addiction: low  
48 sensitivity to aversion and excessive discounting of negative future consequences.  
49 Conversely, the shock-sensitive population may provide a model of humans who are  
50 vulnerable to opportunity loss due to excessive anxiety.

51

52 **Keywords:** punishment, sucrose, compulsivity, addiction, motivation, temporal  
53 discounting, vulnerability, anxiety

54

55           **Introduction**

56           Drug addiction is characterized by excessive motivation to consume the drug  
57 and continued use in spite of negative consequences (Koob and Volkow 2016). The  
58 motivational aspects of addiction and the role of the reward system have been  
59 extensively studied (Wise and Bozarth 1987; Robinson and Berridge 1993; Di Chiara  
60 1999; Koob and Le Moal 2001; Everitt and Robbins 2005; Hyman et al. 2006; Solinas  
61 et al. 2019); however, the investigation of the behavioral and neurobiological  
62 mechanisms underlying the inability to stop drug use in face of adverse consequences  
63 has attracted attention of the field only in the last decade (Vanderschuren and Ahmed  
64 2013; Figeo et al. 2016; Lüscher et al. 2020; Fredriksson et al. 2021; George et al.  
65 2022; McNally et al. 2023). Whereas these processes are intertwined, behavioral and  
66 neurobiological evidence suggest that they depend on partially distinct mechanisms  
67 (Hu 2016; Jean-Richard-Dit-Bressel et al. 2018; McNally et al. 2023).

68           In animal studies, insensitivity to negative outcomes has been mostly  
69 investigated using punishment procedures in which actions leading to a reward are  
70 contingently paired with electrical footshocks (Vanderschuren and Ahmed 2013; Figeo  
71 et al. 2016; Lüscher et al. 2020; Fredriksson et al. 2021; George et al. 2022). This  
72 punishment produces learning processes that result in the reduction of seeking and  
73 taking behaviors (Jean-Richard-Dit-Bressel et al. 2018). Importantly, differences in  
74 sensitivity to punishment have been used as a measure of transition from controlled to  
75 uncontrolled drug use and considered as a marker of addiction (Lüscher et al. 2020).  
76 Obtaining a deeper knowledge of the behavioral and neurobiological basis of  
77 resistance to punishment is critical for better interpretation of results obtained using  
78 these procedures and for understanding the role of punishment processes in  
79 psychiatric disorders such as addiction (Jean-Richard-Dit-Bressel et al. 2018; George  
80 et al. 2022).

81           The emotional impact of events that occur in the future is discounted (i.e. their  
82 perceived value decreases as a function of time) (Chapman 1996; Story et al. 2014;  
83 Bickel et al. 2014). Differences exist among individuals in the rate of discounting, and  
84 individuals who discount reward more rapidly are considered impulsive and at risk to  
85 develop psychiatric disorders such as addiction (Story et al. 2014). Indeed, the  
86 negative consequences of addictive behaviors often occur well after the execution of  
87 the actions leading to the consumption of the object of addiction. Therefore, individuals  
88 that discount punishment too rapidly may not be able to appropriately consider the

89 future consequences of their actions and may be particularly vulnerable to addictive  
90 disorders (Bickel and Marsch 2001; Acuff et al. 2023). Importantly, punishments are  
91 temporally discounted in much the same way that rewards are less effective when  
92 delayed (Bickel et al. 2014). That is, people (Banks and Vogel-Sprott 1965; Estle et al.  
93 2006) and animals (Baron 1965; Woolverton et al. 2012; Rodríguez et al. 2018; Liley  
94 et al. 2019) are more willing to accept negative consequences when they occur after  
95 a delay. Therefore, as previously suggested (Jean-Richard-Dit-Bressel et al. 2018;  
96 George et al. 2022), it is important to investigate the behavioral consequences of  
97 introducing a delay between a reward-seeking action and punishment.

98  
99 We have recently developed a procedure to investigate punishment in rats that,  
100 similarly to what is commonly done in humans (Apergis-Schoute et al. 2017; Kim and  
101 Anderson 2020; Kanen et al. 2021), individually calibrates the strength of punishment  
102 based on the animal's behavior (Desmercieres et al. 2022). This self-adjusting  
103 progressive strength of shock (PSS) procedure allows obtaining PSS break points that  
104 quantify individual resistance to punishment (Desmercieres et al. 2022). Importantly,  
105 compared to other punishment procedures (Panlilio et al. 2003; Deroche-Gamonet et  
106 al. 2004; Pelloux et al. 2007; Krasnova et al. 2014; Bentzley et al. 2014; Pascoli et al.  
107 2015; Datta et al. 2018b; Marchant et al. 2018; Jean-Richard-dit-Bressel et al. 2019;  
108 Durand et al. 2021), the PSS procedure reduces exposure to high levels of shock that  
109 could be particularly aversive and persistently affect operant behavior (Durand et al.  
110 2021), and it therefore could be considered a refinement in the 3Rs (replacement,  
111 reduction, refinement) principles of animal research (Hubrecht and Carter 2019).  
112 Resistance to punishment in this procedure is sensitive to manipulations of motivation  
113 and appears to be both a trait (i.e. PSS break points are highly correlated under a wide  
114 variety of conditions) and a state (i.e. PSS break points are influenced by motivational  
115 states) (Desmercieres et al. 2022). Characterizing behavior in the PSS procedure may  
116 help understanding mechanisms underlying resistance to punishment.

117 In this study, we used the progressive shock strength procedure to investigate  
118 the effects of a range of delays (0-12 sec) on the PSS break points. In brief, after  
119 training in a fixed-ratio 1 (FR 1) food procedure, rats were tested once per week for  
120 resistance to punishment in the PSS procedure or motivation for food in a progressive  
121 ratio (PR) procedure. Each delay was tested at least twice, and at the end of operant  
122 testing, we assessed anxiety-like behavior and pain sensitivity.

123

124 **Material and methods**

125 Forty-eight male Sprague-Dawley rats aged 8-9 weeks (Janvier Labs, France),  
126 experimentally naive at the beginning of the study, were used in this study. The rats  
127 were divided into 2 cohorts of 24 rats which differed in the order of delay presentation.  
128 All experiments were conducted during the light phase and in accordance with  
129 European Union directives (2010/63/EU) for the care of laboratory animals and  
130 approved by the local ethics committees (COMETHEA).

131

132 **Food restriction**

133 During operant procedures and until the end of the experiment, animals  
134 underwent food restriction to limit weight gain and to maintain operant behavior. Food  
135 (approximately 20 g/day) was given 1 hour after the end of the experimental  
136 sessions, and rats had unlimited access to water for the entire duration of experiment.

137

138 **General experimental designs**

139 Fig. S1 shows the experimental design for the two cohorts of rats. After 9 training  
140 sessions under fixed-ratio 1 (FR1) schedules, rats underwent PSS sessions with  
141 varying delays between animals' lever presses and punishment delivery. For the first  
142 3 PSS sessions, on alternate weeks, we also measured responding in the progressive  
143 ratio schedule. Afterwards, rats underwent only PSS test sessions, interspersed with  
144 normal FR1 schedules. The delay was fixed for a given session. Each delay was tested  
145 for 2 to 4 sessions. Tests were performed once per week, every fifth session (normally  
146 on Fridays).

147 Three different delays (3, 6 and 12 sec) were tested. The range of delays was  
148 chosen based on previous work showing that the ability of rats to learn the association  
149 between their behavior and a motivational stimulus depends on the temporal  
150 contingency of the response and the stimulus, and that beyond 8 sec this learning is  
151 impaired (Lattal 2010) and aversion to footshock decreases to about 25% of initial  
152 value in a temporal discounting procedure (Rodríguez et al. 2018).

153 In a first cohort of 24 animals, delays were presented in the following order: 0  
154 sec (x 3 sessions), 3 sec (x2), 12 sec (x3), 6 sec (x3). To rule out an effect of the order  
155 of delays on the effects of shock, delays were presented in a different order in the  
156 second cohort of 24 animals: 6 sec (x2), 3 sec (x2), 0 sec (x2), 12 sec (x2). 24-72h

157 after the last operant sessions, we measured anxiety-related behaviors in an open-  
158 field and pain reactivity in the hot plate.

159 Since behavior did not differ significantly between the two cohorts, we combined  
160 the results.

### 161 **Food Reinforcement Apparatus and training procedure**

162 Experimental chambers (MedAssociates, [www.medassociates.com](http://www.medassociates.com)) were  
163 enclosed individually in sound-attenuation chests. Each experimental chamber had a  
164 recessed food tray, and two levers in the right wall. The floor consisted of bars that  
165 were connected to shockers (MedAssociates, ENV-414SA) that could deliver  
166 footshock, with electric current set to 0.45 mA. Each chamber was equipped with a  
167 food-pellet dispenser, which could deliver 45 mg pellets to the food tray. Experimental  
168 events were controlled by computers using MedAssociates interface and Med-PC IV  
169 software; Med-PC code used to conduct the procedures is available upon request. A  
170 diode light was present on each lever. One lever was assigned to be the active lever  
171 and the corresponding light was used as a conditioned stimulus for food reinforcement.  
172 A third diode light was installed on the opposite wall, and its flashing was used as a  
173 discriminative stimulus to indicate that food reinforcement would be associated with a  
174 foot-shock.

175 The general training schedule involved 45-min sessions of a schedule of food  
176 reinforcement in which each lever press (FR1) produced a 45-mg sucrose pellet.  
177 During these sessions, food availability was signaled by turning off the house-light,  
178 delivery of food was accompanied by flashing of the diode light above the lever for  
179 2 sec. Subsequently, the house light was turned on for an additional 18-sec time-out  
180 period, during which responding had no programmed consequences. Following the  
181 time out, a new trial started and the next response on the right lever was again  
182 reinforced. Responses on the inactive lever were recorded but never reinforced. Rats  
183 initially learned to respond for food during nine sessions under this schedule.

### 184 **Self-adjusting progressive punishment procedure**

185 The self-adjusting progressive shock strength (PSS) procedure was the same  
186 as described by Desmercieres et al (2022). In brief, active lever presses resulted in the  
187 delivery of food rewards and foot-shocks of fixed intensity and variable duration. The  
188 self-adjusting procedure consisted of steps in which the shock duration was increased  
189 if the animal completed 2 trials in the previous step. The duration of the first step was  
190 0 sec (no punishment), the second step was a low duration of 0.05 sec and subsequent

191 shocks increased at each step for 20 steps. The durations of the steps were: 0, 0.05,  
192 0.06, 0.07, 0.08, 0.09, 0.10, 0.12, 0.13, 0.15, 0.18, 0.20, 0.23, 0.27, 0.31, 0.35, 0.41,  
193 0.47, 0.54, 0.62, 0.71 sec. If animals reached the final step, the duration of the shock  
194 was not further increased, and all subsequent shock were set at 0.71 sec. If rats did  
195 not emit any response for 5 min, shock duration was reset to 0 and the shock  
196 progression was reinitialized. The strength of the shock was measured by the electrical  
197 charge in millicoulombs (mC) that an animal was willing to receive to self-administer  
198 food pellets and was calculated by multiplying the fixed current of the shock (0.45 mA)  
199 by the duration in sec. The break point was calculated by the total intensity of the  
200 shocks received during the session. We chose this parameter for the main analysis  
201 because it incorporates both the willingness to receive a given charge unit and the  
202 willingness to restart responding after an eventual punishment-induced pause.

### 203 **Progressive-ratio schedule**

204 Under the progressive-ratio schedule of food reinforcement, the number of  
205 responses required to obtain a food pellet increased with each successive food pellet.  
206 The steps of the exponential progression were the same as those previously  
207 developed by Roberts and colleagues (Richardson and Roberts 1996) adapted for food  
208 reinforcement (Solinas et al. 2003; Solinas and Goldberg 2005), based on the  
209 equation: response ratio =  $(5e^{(0.2 \times \text{reinforcer number})}) - 5$ , rounded to the nearest integer.  
210 Thus, the values of the steps were 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95,  
211 118, 145, 178, 219, 268, 328, 402, 492, 603, and 737. Sessions under the progressive-  
212 ratio schedule lasted until 10 min passed without completing a step, which typically  
213 occurs within 1 h.

### 214 **Anxiety-related behaviors: Open Field**

215 The open-field apparatus (Viewpoint, Lyon, France) consisted of a rectangular  
216 arena (50cm wide \* 50cm long \* 40cm high) of white plexiglass. After a 30-min  
217 habituation to the experiment room, rats were placed in the arena for 30 min. Their  
218 positions were recorded automatically by a camera and video tracking software  
219 (Viewpoint, Lyon, France). The software defined a virtual square (25cm \* 25cm)  
220 delimiting the center zone and the border zone. Anxiety-like behavior was measured  
221 as the percentage (%) of time spent in the center (time in the center/time in the border  
222 + time in the center \* 100) so that more time spent in the center indicated a lower level  
223 of anxiety-like behavior.

### 224 **Pain: Hot plate test**



225 The hot plate (Ugo Basile, model-DS 37) was maintained at 48 °C (Deuis et al.  
226 2017). After a 10-min habituation to the experiment room, animals were placed into a  
227 glass cylinder with 25 cm diameter heated surface and 47 cm walls. The latency before  
228 escape or jumping was recorded. Experiments were stopped after a cut-off of 120s to  
229 prevent unnecessary pain or any tissue damage.

230

### 231 **Statistical Analysis**

232 Med-PC data were analyzed using custom-made, freely available software  
233 written in Python, Med\_to\_csv ([https://github.com/hedjour/med\\_to\\_csv](https://github.com/hedjour/med_to_csv)) that uses raw  
234 data files to create complete tables for further analysis in GraphPad Prism. Data were  
235 checked for normality of distribution using the Shapiro–Wilk test. In our previous study,  
236 in the absence of a delay, PSS data did not show a normal distribution as a function of  
237 the electrical charge variable, but they did after natural logarithmic transformation of  
238 the variable. With the introduction of a delay, PSS data did not show normal distribution  
239 even after logarithmic transformation. Therefore, for statistical analysis, we used a  
240 nonparametric repeated measure Friedman rank test to analyze the effects of different  
241 delays in all rats.

242 To dichotomize individuals into shock sensitive or resistant groups we used the  
243 median split of the PSS break point measured by the total electrical charge sustained  
244 at delay 0 sec. Median splits were obtained in each cohort of rat separately. Whereas  
245 these choices are arbitrary, it should be noted that similar results were obtained when  
246 using PSS break points at 6 or 12 sec of delay or when median splits were calculated  
247 merging the two cohorts.

248 To analyze differences in baseline at the beginning and at the end of the experiment  
249 in all rats and differences in pain sensitivity in shock sensitive or resistant groups, we  
250 used the non-parametric Mann-Whitney test. To analyze the effects of delay in all rats,  
251 we used the non-parametric Friedmann test. For the investigation of the effects of  
252 delays in shock sensitive or resistant groups, we used two-way ANOVA for repeated  
253 measures with (using Geisser-Greenhouse correction when needed to account for  
254 possible violation of sphericity), followed by Sidak's post hoc test. Differences were  
255 considered significant when  $p < 0.05$ .

256

257           **RESULTS**

258           **Food self-administration under basal conditions**

259           Self-administration of food during the entire experiment is shown in Fig. S2 for  
260 the first cohort, and fig.S3 for the second cohort. Rats quickly learned to self-administer  
261 food under FR1 schedules. The initial baseline (BL), calculated as the mean  $\pm$  SEM of  
262 responses per session during the three sessions before the first shock, was  
263  $121.74 \pm 1.53$ , and the final baseline calculated as the average of the last three sessions  
264 was  $122.29 \pm 2.45$ . Statistical analysis using the Mann-Whitney test did not reveal  
265 significant differences between the two baselines ( $p = 0.0643$ ).

266           **Effects of delay on PSS break points**

267           Increasing the delay from 0 to 3 did not produce significant increases in PSS  
268 break point. Only when the delay was further increased to 6 sec did the number of  
269 active responses significantly increase, from about 22 at delay 0 sec to about 48 at  
270 delay 6 and about 51 responses when the delay was set to 12 sec (Fig. 1A). The shock  
271 strength that rats were willing to receive increased from 0.97 to a maximum 8.17 mC  
272 at delay 12 Sec (Fig. 1B). This suggests that delay decreases the magnitude of the  
273 punishment effect. Statistical analysis using the non-parametric Friedman test,  
274 revealed significant differences in both number of responses ( $p < 0.0001$ ) and shock  
275 strength ( $p < 0.0001$ ).

276           **Intra-correlation of PSS break points at different delays and inter-correlation  
277 with PR break points**

278           One of our hypotheses was that individual differences in delay discounting of  
279 aversive consequences could interfere with PSS break points so that animals more  
280 resistant to immediate punishment would not necessarily be more resistant after  
281 introduction of a delay. Contrary to our prediction, correlation among PSS break points  
282 was very high and significant under all conditions (Fig. S4A; Spearman correlation  $R^2$   
283  $> 0.65$  and  $p < 0.0001$  for all values), suggesting that introducing a delay did not alter  
284 the relative sensitivity to punishment.

285 In the first weeks of the experiment, in parallel with PSS we also measured  
286 motivation in a PR procedure (Fig. S1). Consistent with our previous results  
287 (Desmercieres et al. 2022), we found no correlation (Spearman correlation  $R^2 =$   
288  $0.00025$ ,  $p = 0.91$ ) between PSS and PR breakpoints (Fig. S4A-B) confirming that  
289 these tasks do not measure exactly the same behavioral process (Pelloux et al. 2007;  
290 Pascoli et al. 2015; Datta et al. 2018a; Desmercieres et al. 2022).

## 291 **Identification of individuals with high or low resistance to shock-induced** 292 **suppression**

293 Visual analysis of PSS data in fig. 1 reveals that introducing a delay in the PSS  
294 procedures leads to an increase in the variability of behavior among subjects, with  
295 some animals showing increases in PSS with low 3 sec delays and others showing no  
296 or very little increases in the PSS break point even with 12 sec delays. Therefore, we  
297 decided to use a median split of PSS break points at delay 0 sec to classify animals  
298 as shock sensitive and shock resistant and to better characterize these subgroups.  
299 Day-to-day behavior during the entire duration of the procedure is shown in fig. 2A for  
300 cohort 1 and Fig. 2B for cohort 2. A few characteristics deserve to be highlighted. First,  
301 behavior in FR1 training session was very similar in the two groups during initial  
302 training but after repeated shock sessions, the number of responses in the sensitive  
303 groups decreased even on the first training session after the shock, with a slower return  
304 to baseline with repeated training sessions (fig. 2A-2B and fig. S5). This behavior  
305 suggests that shock-sensitive rats develop conditioned suppression of operant  
306 behavior. Second, responding of shock-sensitive and shock-resistant rats were clearly  
307 different in PSS sessions but were similar in PR sessions.

## 308 **Effects of delay in high and low shock-resistant animals**

309 Body weight did not differ between sensitive and resistant animals (sensitive  
310  $375.12 \pm SD 48.14$  g; resistant  $371.99 \pm SD 56.35$  g, Student T-Test:  $P = 0.84$ ) and did  
311 not correlate to PSS break points (Spearman correlation  $R^2 = 0.035$ ,  $p = 0.20$ ).

312 PSS break point as a function of delay in sensitive and resistant animals is depicted in  
313 Fig.3. In both sensitive and resistant rats PSS break point increased with increasing  
314 delays; however, resistant rats showed significant increases with delays as low as 3

315 sec whereas sensitive rats showed increases only at 12 sec of delay. In addition,  
316 resistant rats showed significantly higher break points than sensitive rats at all delays.  
317 Statistical analysis revealed an effect of sensitivity to shock (Responses:  $F(1, 46) =$   
318  $124.4$ ;  $P < 0.0001$ ; Electrical charge:  $F(1, 46) = 42.24$ ;  $P < 0.0001$ ), of delay  
319 (Responses:  $F(2.328, 107.1) = 43.10$ ;  $P < 0.0001$ ; Geisser-Greenhouse's epsilon =  
320  $0.78$ ; Electrical charge:  $F(1.999, 91.95) = 31.32$ ;  $P < 0.0001$ ; Geisser-Greenhouse's  
321 epsilon =  $0.67$ ) and a sensitivity X delay interaction (Responses:  $F(3, 138) = 13.01$ ,  $P$   
322  $< 0.0001$ ; Electrical charge:  $F(3, 138) = 16.13$ ;  $P < 0.0001$ ).

### 323 **Conditioned suppression of operant behavior**

324 As previously noted, after they started to receive shock training, shock-sensitive  
325 animals showed a reduction in the number of responses emitted even in training  
326 sessions in which shock was absent. Therefore, we calculated the difference between  
327 the day before and the day after the PSS session, and we called this measure the  
328 "suppression score". Whereas resistant rats did not show any sign of suppression at  
329 any delay, sensitive rats showed significant conditioned suppression that was similar  
330 at all delays (Fig. 4). Statistical analysis revealed a significant effect of sensitivity to  
331 shock (Responses:  $F(1, 46) = 44.92$ ;  $P < 0.0001$ ), no effect of delay and no significant  
332 sensitivity X delay interaction.

### 333 **Anxiety-like behavior and pain**

334 Difference in sensitivity to shock could be due to trait-like differences in pain  
335 perception or anxiety. To determine whether these parameters influenced behavior, at  
336 the end of the operant sessions, we measured pain in a hot plate test and anxiety-like  
337 behavior in an open field. We found that sensitivity to pain did not differ between  
338 sensitive and resistant rats (Fig. 5A, Mann-Whitney  $U = 258.5$ ,  $P = 0.54$ ) and did not  
339 correlate with PSS breakpoints (Spearman correlation  $R^2 = 0.010$ ,  $p = 0.49$ ; Fig. S6A).  
340 On the other hand, anxiety-like behavior measured at the end of the experiments, was  
341 higher in sensitive compared to resistant rats (Fig. 5B, Mann-Whitney  $U = 164.5$ ,  $P <$   
342  $0.05$ ) and correlated with PSS breakpoints (Spearman correlation  $R^2 = 0.10$ ,  $p = 0.027$ ,  
343 Fig. S6B).

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## **Discussion**

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In this study, we investigated the effects of introducing a delay between reward and punishment in the PSS procedure (Desmercieres et al. 2022). When punishment was delayed, rats were willing to tolerate stronger shocks. Importantly, the introduction of a delay highlighted the existence of two populations of rats: one that not only is resistant to immediate punishment but that also shows stronger discounting of future aversive consequences; and another one that is sensitive to punishment and shows less temporal discounting. Finally, resistance to punishment was not associated with stronger appetitive motivation (as measured by the progressive ratio schedule) or with lower pain sensitivity, but it was negatively correlated with the development of conditioned suppression and anxiety-like behavior.

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In humans, the negative consequences of maladaptive behaviors such as addiction are mostly delayed (Bickel and Marsch 2001; Acuff et al. 2023). This delay contributes to the difficulty in adapting behavior by reducing drug seeking and taking because, similar to what is commonly found with the positive value of rewards (Odum et al. 2020), the negative value of punishment diminishes with the delay (Baron 1965; Banks and Vogel-Sprott 1965; Estle et al. 2006; Woolverton et al. 2012; Rodríguez et al. 2018; Liley et al. 2019). Consistent with those studies, we find that rats were willing to tolerate higher strengths of footshocks when punishment was delayed compared to those tolerated when punishment was immediate. Also, consistent with previous work showing individual differences in delay discounting (Liley et al. 2019), we found considerable differences in the effects of delay on punishment. In fact, the rats that were more resistant to immediate punishment were also those that were more sensitive to temporal discounting. Therefore, using delayed punishment in the PSS procedures provides a method for identifying a population that experiences difficulties in refraining from seeking desired food especially when the negative consequences are not immediate, a key symptom of eating disorders associated with uncontrollable food consumption such as binge-eating disorder and bulimia.

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An unexpected finding in this paper is that shock-sensitive rats not only show a profound reduction in responding on shock days but also a significant reduction on the following days even in the absence of shock. This suppression disappeared and behavior tended to return to baseline only after several sessions without shocks. Thus,

381 the emotional impact of footshock appeared to be a major contributor to the effects of  
382 punishment in this study. Using a procedure that separates pavlovian conditioning from  
383 operant punishment, Jean-Richard-Dit-Bressel and colleagues showed that under  
384 certain conditions, conditioned fear and punishment can be dissociated (Jean-Richard-  
385 dit-Bressel et al. 2019). In contrast, in the PSS procedure, pavlovian and operant  
386 conditioning are not separated, which causes a strong association between  
387 punishment and conditioned suppression. Future studies will be needed to investigate  
388 whether behavior in the PSS procedure and the procedure of Jean-Richard-Dit-Bressel  
389 overlap and whether these procedures identify the same populations of resistant and  
390 sensitive rats.

391 In humans, some individuals could use drugs while managing to maintain  
392 control, but others lose control over their drug-taking and become addicted (Ersche et  
393 al. 2020; Swendsen and Le Moal 2011). However, it is difficult to predict who will  
394 develop addiction and who won't. Animal studies can provide useful information about  
395 the factors that may increase the risks to develop addiction. For drugs, after 20-30  
396 sessions but not earlier, resistance to punishment behavior develops in a minority of  
397 rats (Deroche-Gamonet et al. 2004; Kasanetz et al. 2010). Conversely, in mice models  
398 of food addiction, resistance to punishment using conventional fixed-intensity  
399 procedure did not increase, and it even decreased over time (Domingo-Rodriguez et  
400 al. 2020; García-Blanco et al. 2022). In this study, we tested with the PSS procedure  
401 over long periods (4-6 months) and in a high number of sessions (>80 sessions for  
402 cohort 1 and >60 sessions for cohort 2), and we found no evidence of a progressive  
403 loss of control over food seeking. Indeed, animals identified as shock-sensitive or  
404 shock-resistant after a few sessions continued to show similar behavior for the entire  
405 duration of the experiment. This suggests that at least for food reward, sensitivity to  
406 punishment in the PSS procedure is an individual trait that is not appreciably affected  
407 by repeated exposure to food reward.

408 Previous studies have shown that experience with punishment can profoundly  
409 affect behavior in future situations (Miller 1960; Durand et al. 2021). In particular, early  
410 experience with a high level of shock may induce long-lasting hypersensitivity to shock  
411 and suppress behavior in a persistent manner (Durand et al. 2021). Conversely,  
412 experience with low levels of shock can induce tolerance to punishment and allow  
413 individuals to tolerate higher levels of shocks later on (Miller 1960). The PSS procedure  
414 follows in the latter category. In the present study, changes in PSS were confounded

415 by introduction of delays, but unpublished results in our lab show that upon repeated  
416 exposure, rats reach slightly higher PSS break points compared to initial levels.  
417 However, we found that PSS break-points are highly correlated throughout the  
418 experiments, suggesting that whereas absolute individual levels may increase, relative  
419 resistance to punishment in the PSS procedure is relatively stable over time,  
420 regardless of the order of delays to which animals were exposed. This is a potentially  
421 useful feature of the PSS procedure.

422 Anxiety and addiction are often comorbid psychiatric disorders and their  
423 relationships are bidirectional (Smith and Book 2010). In this study, we found that  
424 anxiety-like behavior measured in the open field was higher in shock -sensitive than in  
425 shock-resistant rats. If we consider shock -resistant rats as addiction-prone, this result  
426 is apparently at odds with previous findings in humans and animals. However, it should  
427 be noticed that we measured anxiety-like behavior at the end of the experiment and  
428 therefore, the results can be affected by previous training in the PSS and could  
429 therefore be the consequence rather than the cause of different behavior in the PSS.  
430 Indeed, anxiety-like behavior in sensitive rats was associated not only with lower PSS  
431 break points but also with higher conditioned suppression, suggesting that repeated  
432 experience of fear in an operant context may have led to diffuse fearful behavior. In  
433 previous studies investigating decision-making when one option is associated with  
434 increasing risks of receiving an electric shock, choice of the risky option was not  
435 associated with anxiety-like behaviors (Simon et al. 2011), even though the anxiolytic  
436 drug diazepam increased risky behavior (Mitchell et al. 2011). More directly relevant  
437 to this study, in a previous study, PSS break point and anxiety-like behavior were not  
438 correlated (Desmercieres et al. 2022). The main difference between that study and this  
439 one is the introduction of delay suggesting that temporal degradation of contingency  
440 induced an anxious phenotype. These results are reminiscent of previous studies in  
441 which probability degradation of punishment contingency produced anxiety-like  
442 behavior (Park and Moghaddam 2017; Jacobs et al. 2022). Importantly, in our study  
443 shock-sensitive rats showed higher anxiety-like behavior than shock-resistant rats,  
444 suggesting that these animals became afraid of their environment even when no real  
445 threat was present.

446 Depending on the circumstances, being relatively resistant or relatively sensitive  
447 to punishment could be either adaptive or maladaptive. In our procedure, we found a  
448 subset of shock-resistant rats that kept seeking food, but they still adapted their

449 behavior depending on the intensity of the shock or the delay. In contrast, the subset  
450 of shock-sensitive rats always stopped responding for food after a few shocks, and  
451 their behavior changed little when the punishment was delayed. In addition, sensitive  
452 animals showed conditioned suppression in the absence of punishment, a behavior  
453 that would not generally be considered optimal in the case of food reward. More  
454 generally, shock-sensitive and shock-resistant animals appear to have different  
455 strategies to cope with environmental challenges. At the individual level, the  
456 conservative (shock-sensitive) strategy may be more adaptive if risks are maintained  
457 and frequent whereas the risk (shock-resistant) strategy may be more adaptive if risks  
458 are temporary and rare.

459 This study has several limitations that should be acknowledged. First, we  
460 investigated the effects of delay only in male rats. Whereas we previously showed that  
461 resistance to punishment for immediate footshock is qualitatively similar between  
462 males and females (Desmercieres et al. 2022), other studies have shown that females  
463 are less sensitive to delay discounting of negative consequences than males (Liley et  
464 al. 2019). In addition, males and females differ in the sensitivity to probabilistic  
465 punishment (Chowdhury et al. 2019). Therefore, it will be important in the future to  
466 investigate the effects of delaying punishment in female rats. Another limitation is that,  
467 to investigate pain sensitivity, we used the hot plate test, a measure of thermal  
468 nociception, whereas our punishment consisted in electrical footshocks. The hot plate  
469 can only provide coarse information about differences in pain sensitivity but it cannot  
470 exclude that subtle or sensory-specific differences would be revealed by more specific  
471 measures of shock-induced pain. However, even though using footshock itself would  
472 allow a more specific measure of the sensitivity to the type of stimulus used as a  
473 punisher, this approach would have clear disadvantages. Indeed, it has been shown  
474 that exposure to shocks could induce tolerance or sensitization to future shocks (Miller  
475 1960; Durand et al. 2021) so that the measure of sensitivity itself and the effect of  
476 punishment may interact and greatly complicate the interpretation of the results. To  
477 avoid this bias and for its simplicity, we have used thermal nociception in the hot plate  
478 as a crude measure of pain sensitivity in punishment procedures as done in similar  
479 previous papers (Degoulet et al. 2021; Li et al. 2021; Desmercieres et al. 2022).

480 In conclusion, introducing a delay between reward and punishment, we  
481 identified a subpopulation of rats that was highly sensitive to shock regardless of delay,  
482 to the extent that their responding was suppressed (and food reward was lost) even



483 when shock was discontinued. These same individuals also showed anxiety-like  
484 behavior in a novel environment and may represent a population vulnerable to  
485 opportunity loss. In contrast, the rest of the rats showed resistance to punishment and  
486 this phenotype was even more pronounced when the consequences of food seeking  
487 were delayed. These individuals represent a population that would be particularly at  
488 risk to develop addiction-like behavior. Thus, the PSS procedure identifies factors that  
489 influence resistance to punishment within a behavioral economics paradigm, and  
490 future work should determine whether it can be used to assess vulnerability to develop  
491 drug addiction.  
492

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505 **Additional Information**

506 Conflict of Interest. The authors declare no competing interests.

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512 Data Availability. The data that support the findings of this study are available from  
513 the corresponding author (MS) upon reasonable request.

514           **References**

- 515       Acuff SF, MacKillop J, Murphy JG (2023) A contextualized reinforcer pathology approach  
516           to addiction. *Nat Rev Psychol* 2:309–323. [https://doi.org/10.1038/s44159-023-](https://doi.org/10.1038/s44159-023-00167-y)  
517           00167-y
- 518       Apergis-Schoute AM, Gillan CM, Fineberg NA, et al (2017) Neural basis of impaired  
519           safety signaling in Obsessive Compulsive Disorder. *Proceedings of the National*  
520           Academy of Sciences 114:3216–3221.  
521           <https://doi.org/10.1073/pnas.1609194114>
- 522       Banks RK, Vogel-Sprott M (1965) Effect of delayed punishment on an immediately  
523           rewarded response in humans. *J Exp Psychol* 70:357–359.  
524           <https://doi.org/10.1037/h0022233>
- 525       Baron A (1965) DELAYED PUNISHMENT OF A RUNWAY RESPONSE. *J Comp Physiol*  
526           Psychol 60:131–134. <https://doi.org/10.1037/h0022326>
- 527       Bentzley BS, Jhou TC, Aston-Jones G (2014) Economic demand predicts addiction-like  
528           behavior and therapeutic efficacy of oxytocin in the rat. *Proceedings of the*  
529           National Academy of Sciences 111:11822–11827.  
530           <https://doi.org/10.1073/pnas.1406324111>
- 531       Bickel WK, Koffarnus MN, Moody L, Wilson AG (2014) The behavioral- and neuro-  
532           economic process of temporal discounting: A candidate behavioral marker of  
533           addiction. *Neuropharmacology* 76:518–527.  
534           <https://doi.org/10.1016/j.neuropharm.2013.06.013>
- 535       Bickel WK, Marsch LA (2001) Toward a behavioral economic understanding of drug  
536           dependence: delay discounting processes. *Addiction* 96:73–86.  
537           <https://doi.org/10.1046/j.1360-0443.2001.961736.x>
- 538       Chapman GB (1996) Temporal discounting and utility for health and money. *Journal of*  
539           Experimental Psychology: Learning, Memory, and Cognition 22:771–791.  
540           <https://doi.org/10.1037/0278-7393.22.3.771>
- 541       Chowdhury TG, Wallin-Miller KG, Rear AA, et al (2019) Sex differences in reward- and  
542           punishment-guided actions. *Cogn Affect Behav Neurosci* 19:1404–1417.  
543           <https://doi.org/10.3758/s13415-019-00736-w>
- 544       Datta U, Martini M, Fan M, Sun W (2018a) Compulsive sucrose- and cocaine-seeking  
545           behaviors in male and female Wistar rats. *Psychopharmacology* 235:2395–2405.  
546           <https://doi.org/10.1007/s00213-018-4937-1>
- 547       Datta U, Martini M, Sun W (2018b) Different functional domains measured by cocaine  
548           self-administration under the progressive-ratio and punishment schedules in  
549           male Wistar rats. *Psychopharmacology* 235:897–907.  
550           <https://doi.org/10.1007/s00213-017-4808-1>

- 551 Degoulet M, Tiran-Cappello A, Combrisson E, et al (2021) Subthalamic low-frequency  
552 oscillations predict vulnerability to cocaine addiction. *Proc Natl Acad Sci USA*  
553 118:e2024121118. <https://doi.org/10.1073/pnas.2024121118>
- 554 Deroche-Gamonet V, Belin D, Piazza PV (2004) Evidence for Addiction-like Behavior in  
555 the Rat. *Science* 305:1014–1017. <https://doi.org/10.1126/science.1099020>
- 556 Desmercieres S, Lardeux V, Longueville J-E, et al (2022) A self-adjusting, progressive  
557 shock strength procedure to investigate resistance to punishment:  
558 Characterization in male and female rats. *Neuropharmacology* 220:109261.  
559 <https://doi.org/10.1016/j.neuropharm.2022.109261>
- 560 Deuis JR, Dvorakova LS, Vetter I (2017) Methods Used to Evaluate Pain Behaviors in  
561 Rodents
- 562 Di Chiara G (1999) Drug addiction as dopamine-dependent associative learning  
563 disorder. *Eur J Pharmacol* 375:13–30. [https://doi.org/10.1016/s0014-](https://doi.org/10.1016/s0014-2999(99)00372-6)  
564 [2999\(99\)00372-6](https://doi.org/10.1016/s0014-2999(99)00372-6)
- 565 Domingo-Rodriguez L, Ruiz De Azua I, Dominguez E, et al (2020) A specific prelimbic-  
566 nucleus accumbens pathway controls resilience versus vulnerability to food  
567 addiction. *Nat Commun* 11:782. <https://doi.org/10.1038/s41467-020-14458-y>
- 568 Durand A, Girardeau P, Freese L, Ahmed SH (2021) Increased responsiveness to  
569 punishment of cocaine self-administration after experience with high  
570 punishment. *Neuropsychopharmacology*. [https://doi.org/10.1038/s41386-021-](https://doi.org/10.1038/s41386-021-01159-3)  
571 [01159-3](https://doi.org/10.1038/s41386-021-01159-3)
- 572 Ersche KD, Meng C, Ziauddeen H, et al (2020) Brain networks underlying vulnerability  
573 and resilience to drug addiction. *Proc Natl Acad Sci USA* 117:15253–15261.  
574 <https://doi.org/10.1073/pnas.2002509117>
- 575 Estle SJ, Green L, Myerson J, Holt DD (2006) Differential effects of amount on temporal  
576 and probability discounting of gains and losses. *Mem Cognit* 34:914–928.  
577 <https://doi.org/10.3758/bf03193437>
- 578 Everitt BJ, Robbins TW (2005) Neural systems of reinforcement for drug addiction: from  
579 actions to habits to compulsion. *Nat Neurosci* 8:1481–1489.  
580 <https://doi.org/10.1038/nn1579>
- 581 Figeo M, Pattij T, Willuhn I, et al (2016) Compulsivity in obsessive–compulsive disorder  
582 and addictions. *European Neuropsychopharmacology* 26:856–868.  
583 <https://doi.org/10.1016/j.euroneuro.2015.12.003>
- 584 Fredriksson I, Venniro M, Reiner DJ, et al (2021) Animal Models of Drug Relapse and  
585 Craving after Voluntary Abstinence: A Review. *Pharmacol Rev* 73:1050–1083.  
586 <https://doi.org/10.1124/pharmrev.120.000191>
- 587 García-Blanco A, Domingo-Rodriguez L, Cabana-Domínguez J, et al (2022) miRNA  
588 signatures associated with vulnerability to food addiction in mice and humans.

- 589 Journal of Clinical Investigation 132:e156281.  
590 <https://doi.org/10.1172/JCI156281>
- 591 George O, Ahmed SH, Gilpin NW (2022) Are we compulsively chasing rainbows?  
592 Neuropsychopharmacol 47:2013–2015. [https://doi.org/10.1038/s41386-022-](https://doi.org/10.1038/s41386-022-01419-w)  
593 01419-w
- 594 Hu H (2016) Reward and Aversion. Annu Rev Neurosci 39:297–324.  
595 <https://doi.org/10.1146/annurev-neuro-070815-014106>
- 596 Hubrecht, Carter (2019) The 3Rs and Humane Experimental Technique: Implementing  
597 Change. Animals 9:754. <https://doi.org/10.3390/ani9100754>
- 598 Hyman SE, Malenka RC, Nestler EJ (2006) Neural mechanisms of addiction: the role of  
599 reward-related learning and memory. Annu Rev Neurosci 29:565–598.  
600 <https://doi.org/10.1146/annurev.neuro.29.051605.113009>
- 601 Jacobs DS, Allen MC, Park J, Moghaddam B (2022) Learning of probabilistic punishment  
602 as a model of anxiety produces changes in action but not punisher encoding in  
603 the dmPFC and VTA. eLife 11:e78912. <https://doi.org/10.7554/eLife.78912>
- 604 Jean-Richard-Dit-Bressel P, Killcross S, McNally GP (2018) Behavioral and  
605 neurobiological mechanisms of punishment: implications for psychiatric  
606 disorders. Neuropsychopharmacology 43:1639–1650.  
607 <https://doi.org/10.1038/s41386-018-0047-3>
- 608 Jean-Richard-dit-Bressel P, Ma C, Bradfield LA, et al (2019) Punishment insensitivity  
609 emerges from impaired contingency detection, not aversion insensitivity or  
610 reward dominance. eLife 8:e52765. <https://doi.org/10.7554/eLife.52765>
- 611 Kanen JW, Apergis-Schoute AM, Yellowlees R, et al (2021) Serotonin depletion impairs  
612 both Pavlovian and instrumental reversal learning in healthy humans. Mol  
613 Psychiatry 26:7200–7210. <https://doi.org/10.1038/s41380-021-01240-9>
- 614 Kasanetz F, Deroche-Gamonet V, Berson N, et al (2010) Transition to addiction is  
615 associated with a persistent impairment in synaptic plasticity. Science 328:1709–  
616 1712. <https://doi.org/10.1126/science.1187801>
- 617 Kim AJ, Anderson BA (2020) The effect of concurrent reward on aversive information  
618 processing in the brain. NeuroImage 217:116890.  
619 <https://doi.org/10.1016/j.neuroimage.2020.116890>
- 620 Koob GF, Le Moal M (2001) Drug addiction, dysregulation of reward, and allostasis.  
621 Neuropsychopharmacology 24:97–129. [https://doi.org/10.1016/S0893-](https://doi.org/10.1016/S0893-133X(00)00195-0)  
622 133X(00)00195-0
- 623 Koob GF, Volkow ND (2016) Neurobiology of addiction: a neurocircuitry analysis. Lancet  
624 Psychiatry 3:760–773. [https://doi.org/10.1016/S2215-0366\(16\)00104-8](https://doi.org/10.1016/S2215-0366(16)00104-8)
- 625 Krasnova IN, Marchant NJ, Ladenheim B, et al (2014) Incubation of Methamphetamine  
626 and Palatable Food Craving after Punishment-Induced Abstinence.

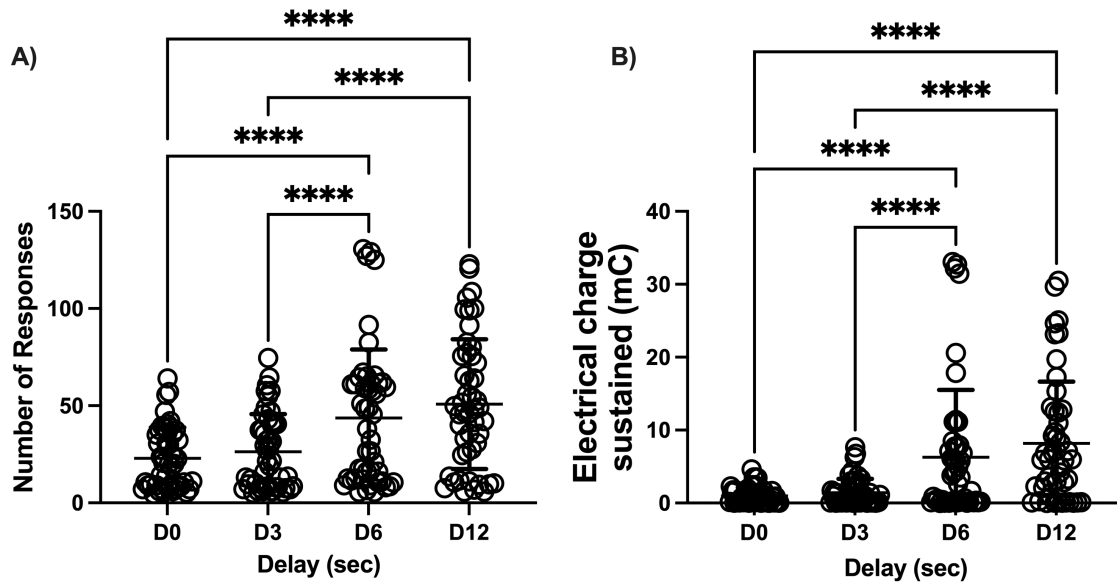
- 627 Neuropsychopharmacology 39:2008–2016.  
628 <https://doi.org/10.1038/npp.2014.50>
- 629 Lattal KA (2010) DELAYED REINFORCEMENT OF OPERANT BEHAVIOR. J Exper Analysis  
630 Behavior 93:129–139. <https://doi.org/10.1901/jeab.2010.93-129>
- 631 Li Y, Simmler LD, Van Zessen R, et al (2021) Synaptic mechanism underlying serotonin  
632 modulation of transition to cocaine addiction. Science 373:1252–1256.  
633 <https://doi.org/10.1126/science.abi9086>
- 634 Liley AE, Gabriel DBK, Sable HJ, Simon NW (2019) Sex Differences and Effects of  
635 Predictive Cues on Delayed Punishment Discounting. eNeuro 6:ENEURO.0225-  
636 19.2019. <https://doi.org/10.1523/ENEURO.0225-19.2019>
- 637 Lüscher C, Robbins TW, Everitt BJ (2020) The transition to compulsion in addiction. Nat  
638 Rev Neurosci 21:247–263. <https://doi.org/10.1038/s41583-020-0289-z>
- 639 Marchant NJ, Campbell EJ, Kaganovsky K (2018) Punishment of alcohol-reinforced  
640 responding in alcohol preferring P rats reveals a bimodal population:  
641 Implications for models of compulsive drug seeking. Progress in Neuro-  
642 Psychopharmacology and Biological Psychiatry 87:68–77.  
643 <https://doi.org/10.1016/j.pnpbp.2017.07.020>
- 644 McNally GP, Jean-Richard-dit-Bressel P, Millan EZ, Lawrence AJ (2023) Pathways to the  
645 persistence of drug use despite its adverse consequences. Mol Psychiatry.  
646 <https://doi.org/10.1038/s41380-023-02040-z>
- 647 Miller NE (1960) Learning resistance to pain and fear: Effects of overlearning, exposure,  
648 and rewarded exposure in context. Journal of Experimental Psychology 60:137–  
649 145. <https://doi.org/10.1037/h0043321>
- 650 Mitchell MR, Vokes CM, Blankenship AL, et al (2011) Effects of acute administration of  
651 nicotine, amphetamine, diazepam, morphine, and ethanol on risky decision-  
652 making in rats. Psychopharmacology 218:703–712.  
653 <https://doi.org/10.1007/s00213-011-2363-8>
- 654 Odum AL, Becker RJ, Haynes JM, et al (2020) Delay discounting of different outcomes:  
655 Review and theory. Journal of the Experimental Analysis of Behavior 113:657–  
656 679. <https://doi.org/10.1002/jeab.589>
- 657 Panlilio LV, Thorndike EB, Schindler CW (2003) Reinstatement of punishment-  
658 suppressed opioid self-administration in rats: an alternative model of relapse to  
659 drug abuse. Psychopharmacology 168:229–235.  
660 <https://doi.org/10.1007/s00213-002-1193-0>
- 661 Park J, Moghaddam B (2017) Risk of punishment influences discrete and coordinated  
662 encoding of reward-guided actions by prefrontal cortex and VTA neurons. eLife  
663 6:e30056. <https://doi.org/10.7554/eLife.30056>

- 664 Pascoli V, Terrier J, Hiver A, Lüscher C (2015) Sufficiency of Mesolimbic Dopamine  
665 Neuron Stimulation for the Progression to Addiction. *Neuron* 88:1054–1066.  
666 <https://doi.org/10.1016/j.neuron.2015.10.017>
- 667 Pelloux Y, Everitt BJ, Dickinson A (2007) Compulsive drug seeking by rats under  
668 punishment: effects of drug taking history. *Psychopharmacology* 194:127–137.  
669 <https://doi.org/10.1007/s00213-007-0805-0>
- 670 Richardson NR, Roberts DC (1996) Progressive ratio schedules in drug self-  
671 administration studies in rats: a method to evaluate reinforcing efficacy. *J*  
672 *Neurosci Methods* 66:1–11. [https://doi.org/10.1016/0165-0270\(95\)00153-0](https://doi.org/10.1016/0165-0270(95)00153-0)
- 673 Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-  
674 sensitization theory of addiction. *Brain Res Brain Res Rev* 18:247–291.  
675 [https://doi.org/10.1016/0165-0173\(93\)90013-p](https://doi.org/10.1016/0165-0173(93)90013-p)
- 676 Rodríguez W, Bouzas A, Orduña V (2018) Temporal discounting of aversive  
677 consequences in rats. *Learn Behav* 46:38–48. [https://doi.org/10.3758/s13420-](https://doi.org/10.3758/s13420-017-0279-9)  
678 [017-0279-9](https://doi.org/10.3758/s13420-017-0279-9)
- 679 Simon NW, Montgomery KS, Beas BS, et al (2011) Dopaminergic Modulation of Risky  
680 Decision-Making. *J Neurosci* 31:17460–17470.  
681 <https://doi.org/10.1523/JNEUROSCI.3772-11.2011>
- 682 Smith JP, Book SW (2010) *Anxiety and Substance Use Disorders: A Review*
- 683 Solinas M, Belujon P, Fernagut PO, et al (2019) Dopamine and addiction: what have we  
684 learned from 40 years of research. *J Neural Transm (Vienna)* 126:481–516.  
685 <https://doi.org/10.1007/s00702-018-1957-2>
- 686 Solinas M, Goldberg SR (2005) Motivational effects of cannabinoids and opioids on food  
687 reinforcement depend on simultaneous activation of cannabinoid and opioid  
688 systems. *Neuropsychopharmacology* 30:2035–2045.  
689 <https://doi.org/10.1038/sj.npp.1300720>
- 690 Solinas M, Panlilio LV, Antoniou K, et al (2003) The Cannabinoid CB1 Antagonist *N*-  
691 Piperidinyl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-  
692 carboxamide (SR-141716A) Differentially Alters the Reinforcing Effects of Heroin  
693 under Continuous Reinforcement, Fixed Ratio, and Progressive Ratio Schedules  
694 of Drug Self-Administration in Rats. *J Pharmacol Exp Ther* 306:93–102.  
695 <https://doi.org/10.1124/jpet.102.047928>
- 696 Story G, Vlaev I, Seymour B, et al (2014) Does temporal discounting explain unhealthy  
697 behavior? A systematic review and reinforcement learning perspective. *Frontiers*  
698 *in Behavioral Neuroscience* 8:76. <https://doi.org/10.3389/fnbeh.2014.00076>
- 699 Swendsen J, Le Moal M (2011) Individual vulnerability to addiction. *Annals of the New*  
700 *York Academy of Sciences* 1216:73–85. [https://doi.org/10.1111/j.1749-](https://doi.org/10.1111/j.1749-6632.2010.05894.x)  
701 [6632.2010.05894.x](https://doi.org/10.1111/j.1749-6632.2010.05894.x)

- 702 Vanderschuren LJMJ, Ahmed SH (2013) Animal Studies of Addictive Behavior. Cold  
703 Spring Harb Perspect Med 3:. <https://doi.org/10.1101/cshperspect.a011932>
- 704 Wise RA, Bozarth MA (1987) A psychomotor stimulant theory of addiction. Psychol Rev  
705 94:469–492
- 706 Woolverton WL, Freeman KB, Myerson J, Green L (2012) Suppression of cocaine self-  
707 administration in monkeys: effects of delayed punishment. Psychopharmacology  
708 (Berl) 220:509–517. <https://doi.org/10.1007/s00213-011-2501-3>
- 709



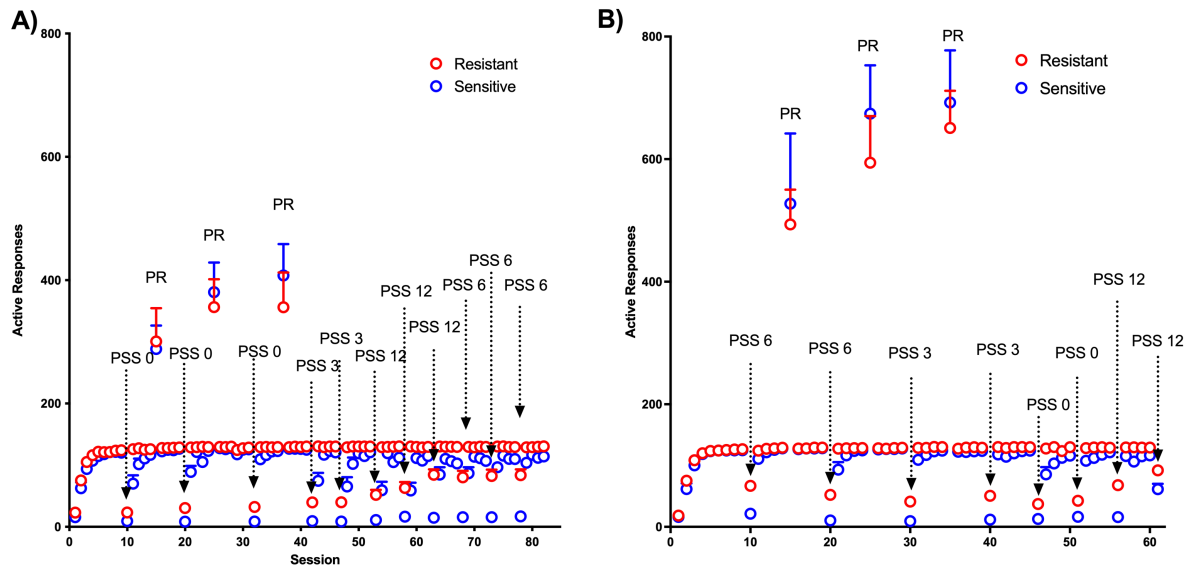
710 **Figure and Legends**



711

712 **Fig. 1. Effect of delay on resistance to punishment.** Number of active responses  
713 (A) and PSS break point (total electrical charge sustained) (B) as a function of the  
714 delay between the response (and the food delivery) and the footshock. Data are  
715 expressed as mean  $\pm$  SD of active responses (N = 48). Each data point corresponds  
716 to the average of at least 2 sessions at a given delay. Friedman nonparametric post-  
717 hoc test for repeated measures: \*\*\*\*,  $P < 0.0001$ .

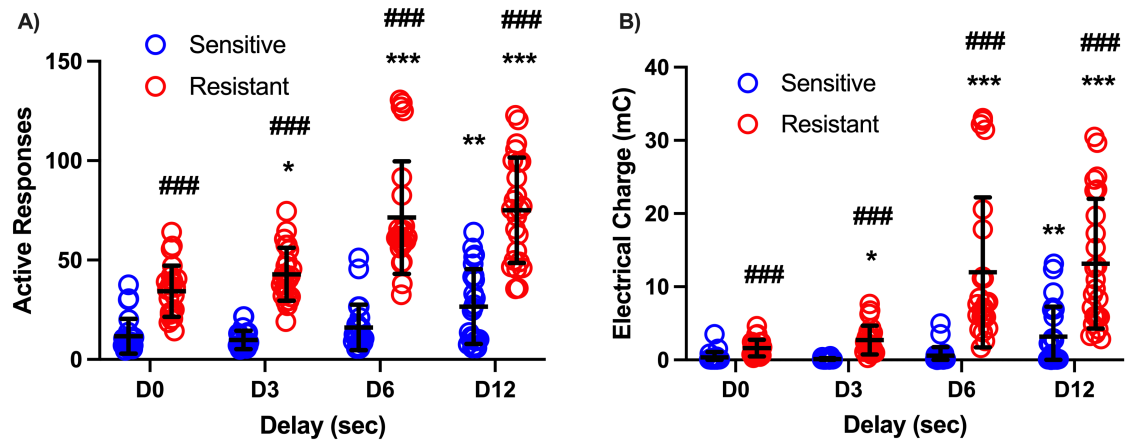
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721 **Fig. 2. Operant behavior in shock sensitive and shock resistant rats during the**  
 722 **entire experiment.** Number of active responses in all the 84 sessions of the  
 723 experiment for A) cohort 1 and B) cohort 2. It should be noticed that during normal  
 724 training and shock sessions each active responses produces the delivery of one  
 725 sucrose pellet whereas in PR sessions each subsequent pellet requires an increasing  
 726 number of responses. N = 12 per group.

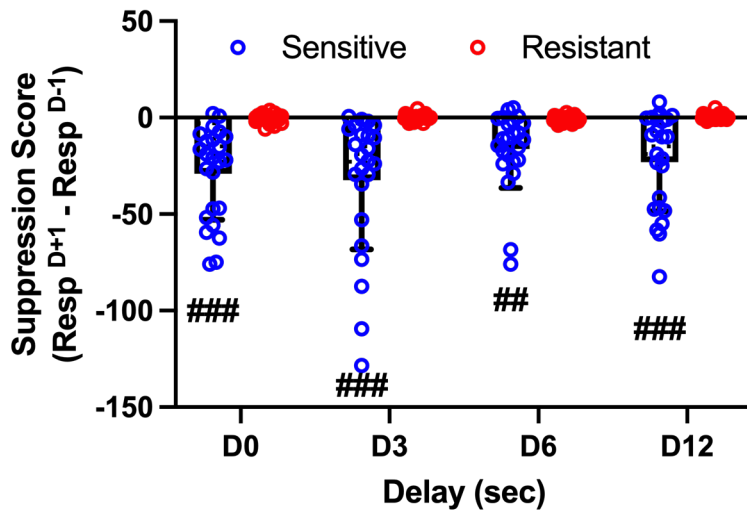
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729 **Fig. 3. PSS break points as a function of the delay in shock-sensitive vs shock-**  
 730 **resistant rats.** PSS break point measured as the number of active responses (A) and  
 731 total electrical charge sustained (B) as a function of the delay between the response  
 732 (and the food delivery) and the footshock. Data are expressed as mean  $\pm$  SD of active  
 733 responses (Males  $n = 24$  per group). Each data point corresponds to the average of at  
 734 least 2 sessions at a given delay. Notice that median split was calculated separately  
 735 in each cohort of rat which results in some overlapping of sensitive and resistant rats  
 736 at delay 0. Two-way ANOVA for repeated measures: \*, \*\*, \*\*\* =  $P < 0.5$ ,  $P < 0.001$  and  
 737  $P < 0.0001$  compared to Delay 0 sec (D0); #### =  $P < 0.0001$  compared to shock  
 738 sensitive rats.

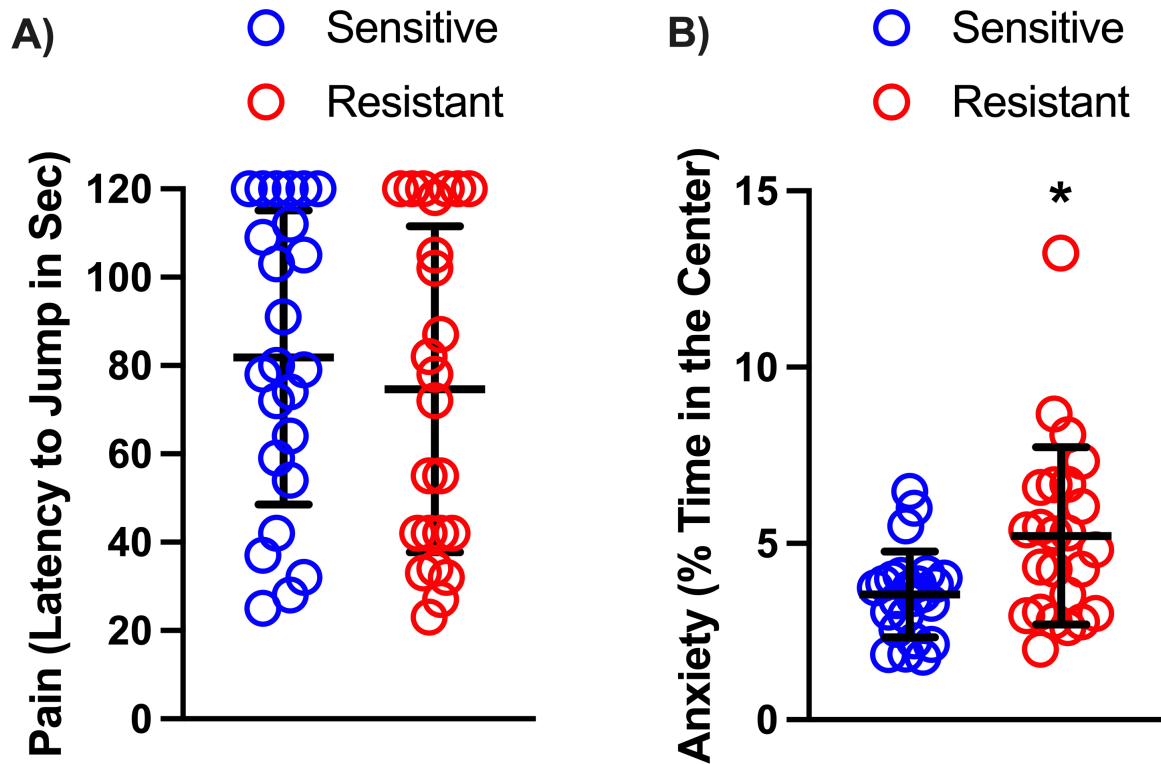
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741 **Fig. 4. Conditioned suppression as a function of the delay in shock sensitive vs**  
 742 **shock resistant rats.** The suppression score was calculated as the difference in the  
 743 number of active responses between the day after (D+1) and the day before (D-1) the  
 744 shock session. Data are expressed as mean ± SD of active responses (Males n = 24  
 745 per group). Two-way ANOVA for repeated measures: ##, ### = P < 0.001, P < 0.0001  
 746 compared to shock resistant rats.

747



748

749 **Fig. 5. Pain sensitivity and anxiety-like behavior in shock sensitive vs shock**  
 750 **resistant rats.** Pain sensitivity in the hot plate test (A) and anxiety-like behavior in an  
 751 open field (B). These tests were performed at the end of the experiment. Note that the  
 752 rats that spend more time in the center of the open field, are considered the less  
 753 anxious. Data are expressed as mean ± SD of active responses (Males n = 24 per  
 754 group). Mann-Whitney test: \* = P < 0.05, P < 0.0001.