

1 **Optogenetic interrogation of the role of striatal patches in habit** 2 **formation and inhibition of striatal dopamine**

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15 16 **Abstract**

17 Habits are inflexible behaviors that can be maladaptive in diseases including drug addiction. The
18 striatum is integral to habit formation, and interspersed throughout the striatum are patches, or
19 striosomes, which are characterized by unique gene expression relative to the surrounding matrix.
20 Recent work has indicated that patches are necessary for habit formation, but how patches
21 contribute to habits remains partially understood. Here, using optogenetics, we modulated striatal
22 patches in Sepw1-NP67 mice during habit formation. We find that patch activation during operant
23 training impairs habit formation, and conversely, that acute patch stimulation after reward
24 devaluation can drive habitual reward seeking. Patch stimulation invigorates general locomotion
25 but is not inherently rewarding. Finally, we use fast-scan cyclic voltammetry to demonstrate that
26 patch stimulation suppresses dopamine release in dorsal striatum *in vivo*. Overall, this work
27 provides novel insight into the role of the patch compartment in habit formation, and potential
28 interactions with dopamine signaling.

29 Introduction

30 Organisms must optimize action patterns to be successful in their environments. This
31 optimization process can come in two forms: updating of actions can be highly flexible and
32 dependent on outcomes (so-called action-outcome, or goal-oriented behaviors) or, with extended
33 training, action updating can become resistant to change regardless of outcome (stimulus-response
34 or habitual behaviors; Dolan and Dayan, 2013). Habitual, automated behaviors can be highly
35 advantageous, as they allow animals to respond to stimuli without great cognitive effort. However,
36 habits can also present as maladaptive behaviors that persist in spite of negative outcomes.
37 Moreover, dysfunctional habit formation underlies many pathological states, including drug
38 addiction (Robbins and Everitt, 1999).

39 In animal models, habits have been studied by measuring perseverance of instrumental
40 behaviors following reduction in reward value or by measuring flexibility when action-outcome
41 contingencies are manipulated (Adams and Dickinson, 1981; Dickinson, 1985; Rossi and Yin,
42 2012). Using these approaches, distinct neural circuits underlying goal-directed and habitual
43 responding have been identified. A well supported model has emerged positing that the
44 dorsomedial striatum encodes goal-directed behaviors, while the dorsolateral striatum encodes
45 habitual behaviors (Yin et al., 2005, 2004; Yin and Knowlton, 2006). Similarly, corticostriatal
46 plasticity in the lateral striatum correlates with habitual responding (O'Hare et al., 2016), and
47 human imaging studies have linked activity in lateral striatum (putamen) with habitual behaviors
48 (Tricomi et al., 2009). However, this model could be somewhat oversimplified, as other studies
49 suggest medial striatum could also contribute to inflexible behaviors (Malvaez et al., 2018; Seiler
50 et al., 2020).

51 Adding a layer of complexity to the medial-lateral striatal divide is the existence of two
52 neurochemically distinct subcompartments: small, labyrinthine islands called patches or
53 striosomes (comprising 15% of striatal volume), and surrounding 'matrix' tissue (85% of striatal
54 volume; Gerfen, 1992; Graybiel and Ragsdale, 1978). In addition to unique cellular markers
55 (Crittenden and Graybiel, 2011), patches are characterized by unique connectivity, providing the
56 predominant anatomical and functional striatal input to midbrain dopaminergic neurons (Evans et
57 al., 2020; Gerfen, 1985). Additionally, habenula-projecting neurons of the entopeduncular nucleus
58 receive preferential input from patches (Stephenson-Jones et al., 2016; Wallace et al., 2017).
59 Striatal patches also have unique input profiles, with preferential inputs from frontal cortex (Eblen
60 and Graybiel, 1995; Gerfen, 1984; but see Smith et al., 2016). Therefore, striatal patches are well-
61 positioned to serve as a limbic-motor interface that could subserve action selection (Shivkumar et
62 al., 2017).

63 Despite extensive work characterizing the structure and connectivity of striatal patches,
64 their role in behavior regulation is only partially understood. Studies have suggested a role for
65 striatal patches in reward processing (Bloem et al., 2017; White and Hiroi, 1998; Yoshizawa et al.,
66 2018) and cost-benefit decision making (Friedman et al., 2017, 2015). Additionally, several studies
67 now support the notion that patches may encode the transition from flexible to habitual responding.
68 Early studies suggested that psychostimulant-induced stereotypy is linked to activity in patches

69 (Canales and Graybiel, 2000; Murray et al., 2015, 2014) and that lesions of patches disrupt this
70 stereotypy (Murray et al., 2015, 2014). More recently, striatal patches have been shown to be
71 necessary for normal habit formation: specific lesions of patch neurons diminish habitual
72 responding following reward devaluation (Jenrette et al., 2019) or changes in action-outcome
73 contingencies (Nadel et al., 2020).

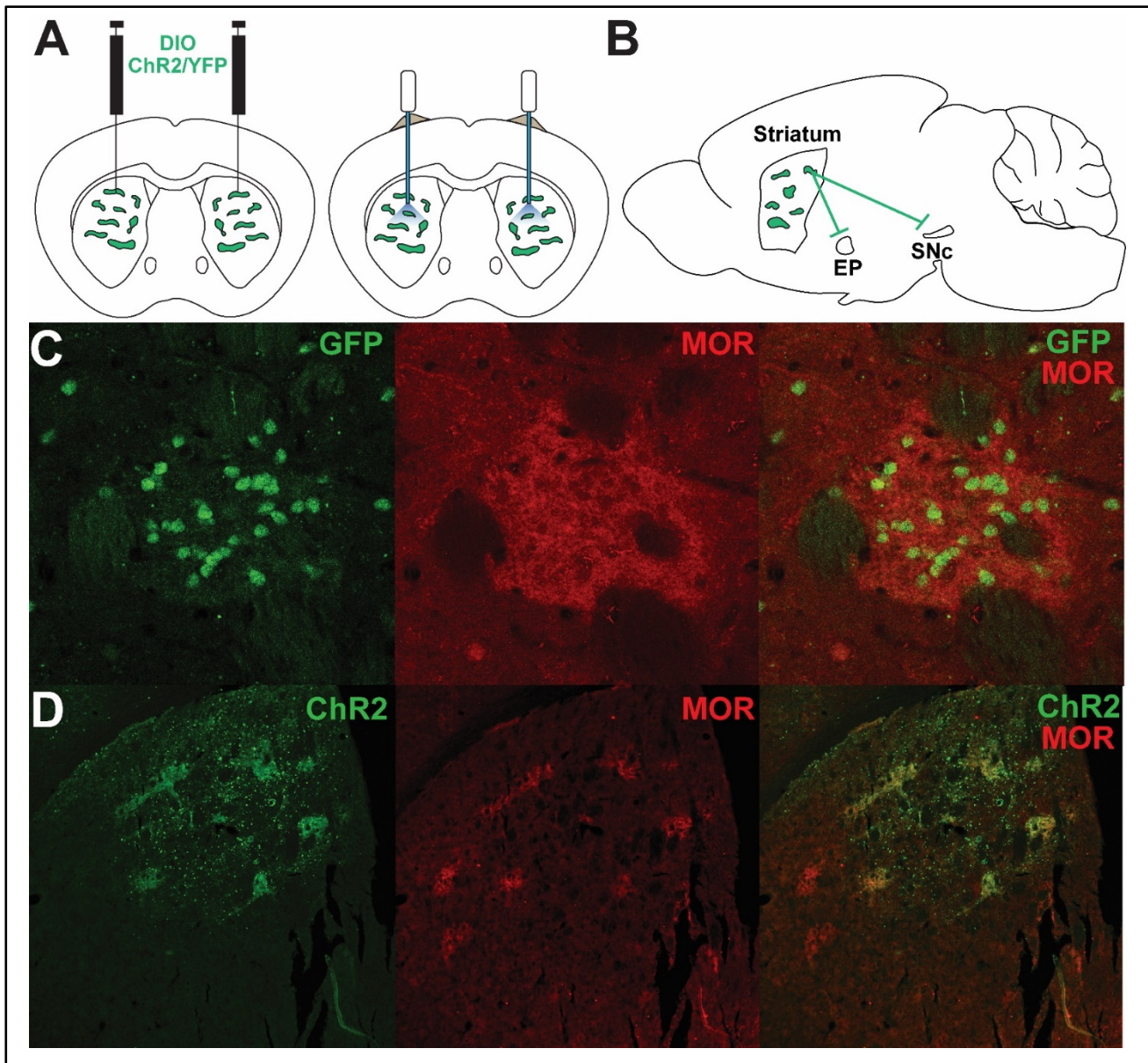
74 In the current study, we employed optogenetics in Sepw1-NP67 mice with enriched Cre
75 recombinase expression in striatal patches (Gerfen et al., 2013) to selectively target patches.
76 Patches or patch projections were stimulated at reward retrieval during a variable interval schedule
77 of responding, a task used to induce habitual responding (Gremel and Costa, 2013; Rossi and Yin,
78 2012). Mice that received stimulation of striatal patches reduced lever pressing and head entry
79 rates to a greater extent than YFP controls following reward devaluation, implying impaired habit
80 formation. Following retraining and subsequent reward devaluation, acute stimulation of patches
81 was sufficient to drive habitual reward seeking behaviors. Contrary to a prior study using non-
82 selective electrical self-stimulation (White and Hiroi, 1998) we did not find optogenetic
83 stimulation of patches to be reinforcing in a place preference task, but stimulation of patches did
84 elevate locomotion in an open field. Finally, to investigate how patch activation modifies circuit
85 function, we employed fast-scan cyclic voltammetry to measure striatal dopamine levels *in vivo*
86 and determined that optogenetic activation of patches suppresses dopamine release driven by
87 electrical stimulation of excitatory inputs. Together, these results suggest striatal patches are a key
88 site underlying habit formation and that activating patches can drive habitual reward seeking,
89 potentially by modulating striatal dopamine levels.

90

91 **Results**

92 **Optogenetic manipulation of striatal patches or projections in variable interval training**

93 To investigate the role of patch neurons in habit formation, we utilized Sepw1-NP67 mice,
94 which have enriched Cre recombinase expression in striatal patches (Gerfen et al., 2013; Smith et
95 al., 2016). Crossing these mice with a Cre-dependent GFP reporter line shows enriched GFP+
96 neurons in μ -opiate receptor dense striatal patches (Crittenden and Graybiel, 2011, Figure 1C),
97 though as previously reported, this line also expressed Cre in “exo-patch” neurons, which display
98 similar gene expression and physiological profiles to patch neurons (Smith et al., 2016). We
99 injected Sepw1-NP67 mice with an AAV encoding either Cre-dependent light-gated cation
100 channel ChR2 or YFP in the dorsal striatum, which resulted in enriched ChR2 expression in striatal
101 patches (Figure 1D). We then implanted fiber optics targeting cell bodies of striatal patch neurons,
102 patch terminals in SNc (Evans et al., 2020), or at patch terminals in entopeduncular nucleus
103 (Stephenson-Jones et al., 2016; Wallace et al., 2017; Figure 1A + B) with the expectation that these
104 two pathways may differentially modulate habitual responding due to potentially opposing effects
105 on dopamine neurons. However, no implantation site-dependent differences were observed in
106 performance during training, habit probes, open field, or place preference tasks ($p > 0.05$),
107 therefore fiber optic placement groups were collapsed into a general “ChR2” group for comparison
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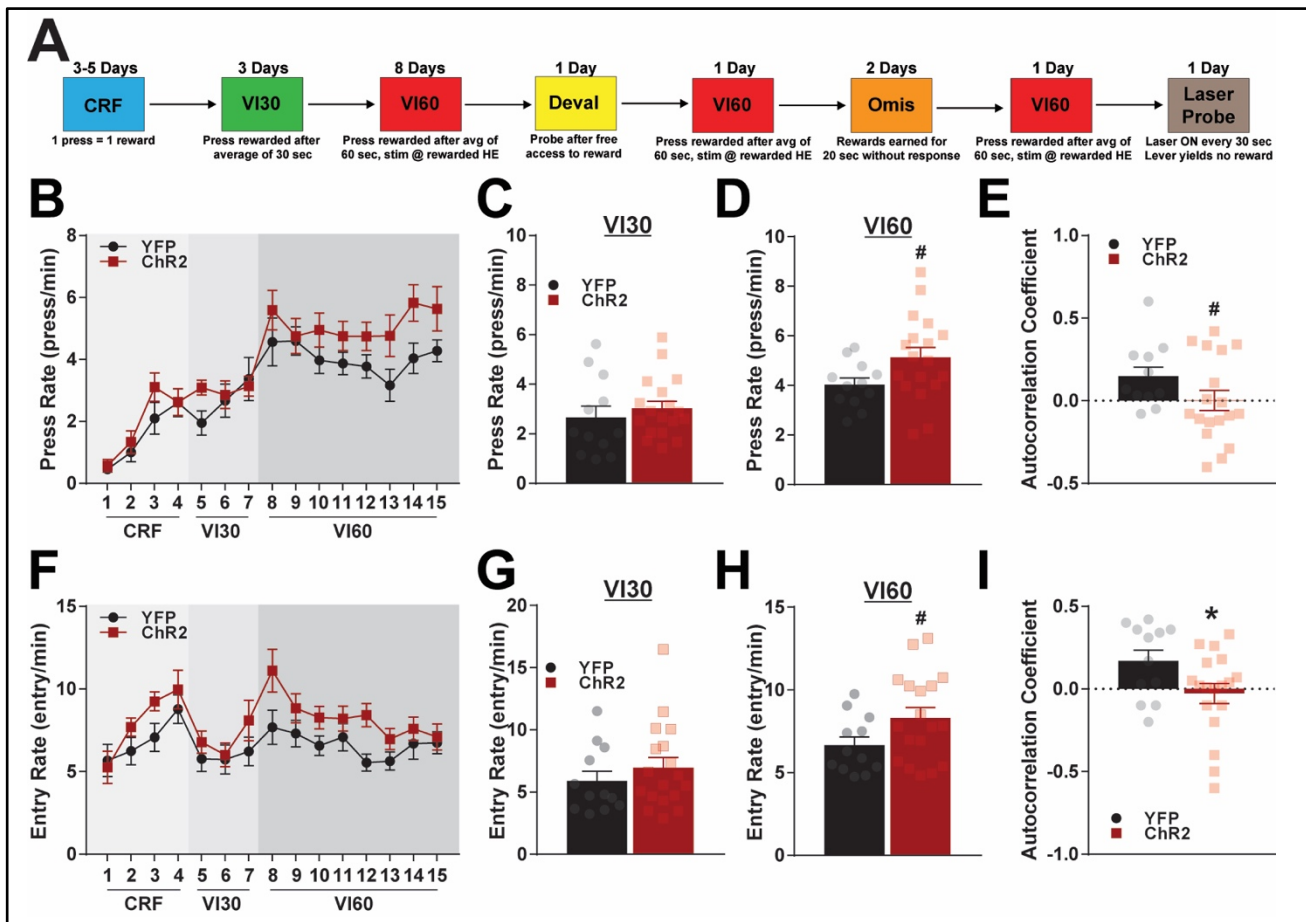
111 **Figure 1. Experimental Design and Characterization of Sepw1-Cre Mice.** A. Experimental design. Bilateral AAV
112 driving expression of channelrhodopsin-2 (ChR2) or YFP was injected into dorsal striatum (left). Fiber optics were
113 affixed just dorsal to injection site (right). B. Patches in striatum, or patch projections to entopeduncular nucleus (EP)
114 or substantia nigra pars reticulata were targeted with fiber optic implants. C. Coronal section showing Cre dependent
115 expression of GFP and μ -opioid receptor expression demonstrating enriched Cre expression in a striatal patch. D.
116 Coronal section showing AAV5-driven expression of ChR2-eYFP overlaid with μ -opioid receptor expression.

117

118 with YFP controls (individual group data is shown in supporting figures matching main figure
119 numbers; Supporting Figures 2-5).

120 Three weeks after surgery, mice were food restricted and trained to depress a lever on a
121 continuous reinforcement schedule (CRF), a variable interval averaging 30 sec (VI30), then a
122 variable interval averaging 60 sec (VI60) schedule of reinforcement (see Figure 2A for behavioral
123 schedule), which induces habitual behavior in mice (Rossi and Yin, 2012). Beginning in VI60,

124 mice received laser stimulation through fiber optics at reward retrieval (first headentry following
 125 reward delivery; 3 sec, 5 Hz, 5 mW stimulation). Both ChR2 and YFP mice increased press rates
 126 across CRF and VI training (two-way repeated measures ANOVA, significant effect of time, $F_{(14, 392)} = 20.04$, $p < 0.0001$). However, ChR2-stimulated mice had a tendency to press at a slightly
 127 higher rate across training (trending effect of group, $F_{(1, 28)} = 3.841$, $p = 0.060$; no significant
 128 interaction, $p > 0.05$; Figure 2B). This phenomenon was not attributable to differences during VI30
 130 training ($t_{28} = 0.7205$, $p = 0.477$; Figure 2C), but rather a tendency for ChR2 mice to increase
 131 pressing following the onset of stimulated trials in VI60 ($t_{28} = 2.013$, $p = 0.054$; Figure 2D). We
 132 previously found that caspase-driven lesions of striatal patches increased response variability
 133 across days (Nadel et al., 2020), and similarly, optogenetic activation of patches during VI60
 134 training had a tendency to reduce press rate consistency as assessed by day-to-day autocorrelation
 135 (lag 1 day; unpaired t-test, $t_{28} = 1.691$, $p = 0.102$; Figure 2E).
 136



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 138
 139 **Figure 2. Optogenetic patch stimulation during variable interval training.** A. Timeline of behavioral schedule. Mice were trained on a continuous reinforcement schedule (CRF) before beginning variable interval 30 training (VI30). Following this, mice began a variable interval 60 schedule (VI60), and optogenetic stimulation of patches or patch projections occurred during reward retrieval. Mice then experienced reward devaluation before being retrained on VI60. Mice then experienced and two days of omission (Omis). Finally, following retraining on VI60, mice experienced a “Laser Probe” trial. See Methods for details of each behavioral schedule. B. Press rates for ChR2 and

Figure 2 continued on next page

145 YFP control mice across training. C-D. Average response rates across VI30 (C) or VI60 (D). E. Autocorrelation
146 coefficients for press rates across VI60 training. F. Head entry rates to the food magazine across training for ChR2
147 and YFP mice. G-H. Average head entry rates across VI30 (G) and VI60 (H). I. Autocorrelation coefficients for entry
148 rates across VI60 training. * $P < 0.05$; # $P < 0.1$; error bars, SEM.

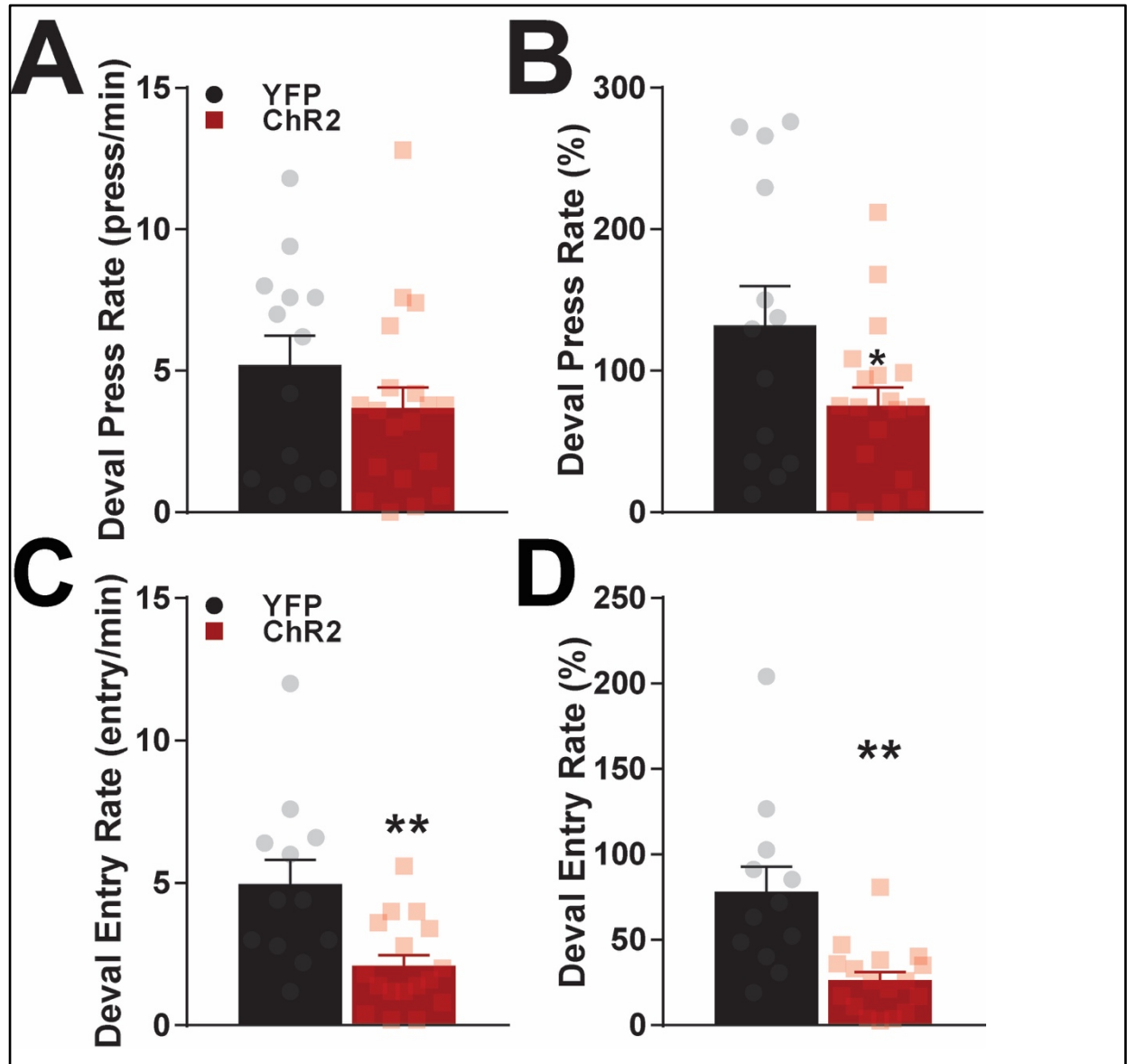
149
150 In addition to lever pressing, head entry to the food magazine has been used to assess
151 flexibility (DePoy et al., 2016; Morrison et al., 2015; Sieburg et al., 2019). ChR2 mice and YFP
152 controls both altered their entry rates across training (two-way repeated measures ANOVA,
153 significant effect of time, $F_{(14, 392)} = 5.148$, $p < 0.0001$), though ChR2 mice tended to have higher
154 entry rates (trending effect of group, $F_{(1, 28)} = 3.204$, $p = 0.0843$; no significant interaction, $p >$
155 0.05 ; Figure 2F). This was also not attributable to VI30 ($t_{28} = 0.8953$, $p = 0.3782$ Figure 2G), but
156 a trending increase in entry rate was noted during stimulated VI60 trials ($t_{28} = 1.922$, $p = 0.0649$;
157 Figure 2H). Additionally, activating patches across VI60 training resulted in even clearer
158 disruption of day-to-day consistency in head entries as assessed by autocorrelation (lag 1 day;
159 unpaired t-test, $t_{28} = 2.145$, $p = 0.0407$; Figure 2I). Taken together, this data suggests that ChR2
160 injections do not impair learning in CRF or VI30, but that stimulation of patches slightly
161 invigorates responding in VI60 when stimulation is paired to rewarded head entries. Further, this
162 data suggests that modulation of patches impairs day-to-day response consistency.

163

164 **Characterizing patches stimulation during learning in devaluation and omission probes**

165 Habits are operationally defined as behaviors resistant to outcome devaluation. Therefore,
166 after the completion of eight VI60 training days, mice received free access to sucrose for an hour
167 before being returned to behavioral chambers for a 5 min devaluation probe trial. Visual
168 confirmation was made to ensure each mouse drank sucrose during free access, and the weight of
169 sucrose consumed was not significantly different between implantation sites (Fig, S3A; $p > 0.05$)
170 or between ChR2 and YFP mice (Figure S3B; $p > 0.05$) in a subgroup of mice. During probe trials,
171 lever presses and head entries were recorded, but no rewards were delivered. ChR2 and YFP mice
172 did not significantly differ in raw press rate during devaluation (unpaired t-test, $t_{28} = 1.382$, $p =$
173 0.178 ; Figure 3A). However, due to the increased variability in ChR2 mice and slightly different
174 press rates between groups, we normalized the devaluation press rate to mean press rates across
175 VI60 for each mouse. ChR2 mice pressed significantly less in the devaluation probe when
176 normalized to baseline responding, indicating weaker habit formation (unpaired t-test, $t_{28} = 2.261$,
177 $p = 0.0317$; Figure 3B). Similarly, ChR2 mice entered the reward port less frequently than YFP
178 controls during devaluation probes, both in raw ($t_{27} = 3.398$, $p = 0.0021$; Figure 3C) and normalized
179 entry rate ($t_{27} = 3.845$, $p = 0.0007$; Figure 3D).

180 One day after devaluation probes, mice were retrained on a VI60 schedule with optogenetic
181 stimulation of patches to reinstate robust pressing before beginning two days of omission probes.
182 In omission, mice were required to abstain from pressing for 20s in order to receive a reward. This
183 probe has been used as an alternative means to assess habit by measuring flexibility in forming
184 new action-outcome contingencies (Yin et al., 2005, 2004), and we previously reported that mice
185 with patch lesions have reduced pressing in omission trials. However, we did not observe any



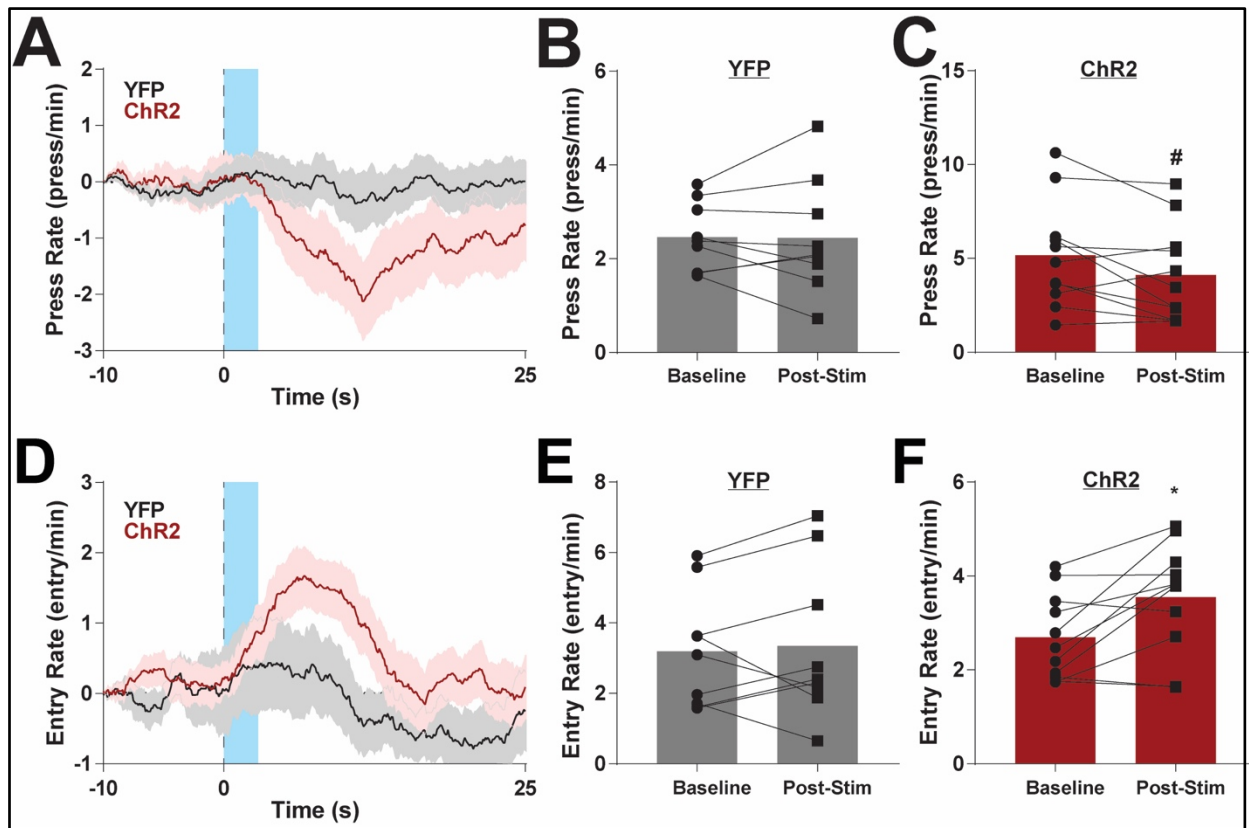
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188 **Figure 3. Effects of optogenetic patch manipulation in learning following devaluation.** A. Average press rates
189 during devaluation probe trials (see Methods). B. Average press rates in devaluation probes normalized to baseline
190 responding during VI60 training. C. Average head entry rates during devaluation probe trials. D. Average head entry
191 rates in devaluation probes normalized to baseline entry rate during VI60 training. * $P < 0.05$; error bars, SEM.
192

193 effect of ChR2 stimulation on raw or normalized press or entry rates relative to controls
194 (Supporting Figure 1A-F, all $p > 0.05$). As expected, YFP mice had a strong correlation between
195 press rate on day 1 of omission and press rate on the reinstatement day between devaluation and
196 omission probes (Pearson's correlation, $R^2 = 0.551$, $p = 0.0057$; Supporting Figure 1G). However,
197 ChR2 mice did not display any correlation between press rates during these days ($R^2 = 0.0095$, p
198 $= 0.6993$; Supporting Figure 1H). This finding could further suggest impaired day-to-day
199 consistency in responding in patch stimulated mice.
200

201 **Determining acute effects of optogenetic stimulation of patches following devaluation**

202 If patches encode habits, we reasoned that acute optogenetic stimulation of patches may
203 drive habitual responding even following reward devaluation. Mice therefore underwent another
204 day of VI60 retraining with optogenetic stimulation to reinstate robust pressing. Following this,
205 mice began a novel probe trial, which began with one hour of free access to sucrose to devalue
206 rewards. Mice were then placed in the operant chamber with the lever extended, though no rewards
207 were delivered. Laser stimulation was delivered on a variable 60 sec interval, which was not
208 contingent on responding, and trials lasted 30 min (referred to as a “laser probe” trials). Press rates
209 for YFP and ChR2 are shown relative to laser onset (blue) in Figure 4A. Laser stimulation did not
210 alter pressing behaviors in YFP mice (paired t-test, $t_8 = 0.0622$, $p = 0.9519$; Figure 4B). To our
211 surprise, laser stimulation in ChR2 mice was immediately followed by a near-significant decrease
212 in lever pressing ($t_{10} = 2.193$, $df = 10$, $p = 0.0531$; Figure 4C). To investigate what happens during
213 this decrease in pressing, we repeated this analysis focusing on head entries before and after
214 stimulation (Figure 4D). Again, stimulation in YFP controls did not alter entry rate ($t_8 = 0.4415$, p
215 $= 0.6705$), however, ChR2 immediately increased entry rate following stimulation ($t_{10} = 3.049$, p
216 $= 0.0123$). These data suggest that patch stimulation drives habitual reward seeking following
217 reward devaluation.



218 **Figure 4. Effects of acute optogenetic patch manipulation in laser probe trials.** A. Average baseline normalized
219 press rates before and after laser onset. Laser stimulation (5Hz, 3 sec) is indicated by blue. B-C. Press rates before and
220 after laser onset for YFP (B) or ChR2 (C) mice. D. Average baseline normalized head entry rates before and after laser
221 stimulation. E-F. Head entry rates before and after laser onset for YFP (E) or ChR2 (F) mice. # indicates a significant
222 decrease in press rate, * indicates a significant increase in entry rate.

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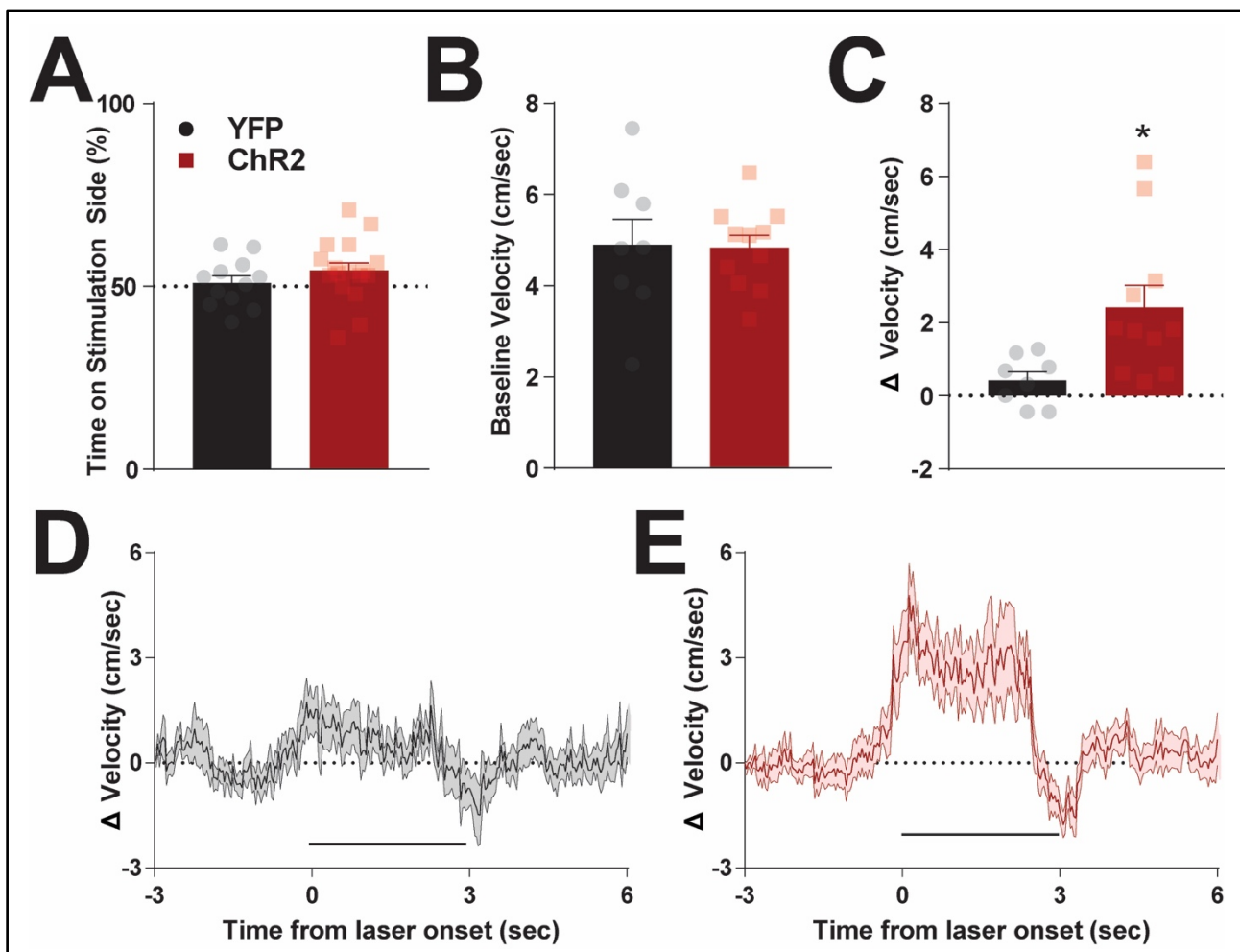
223 onset. E-F. Head entry rates before and after laser onset for YFP (E) or ChR2 (F) mice. * $P < 0.05$; # $P < 0.1$; error
224 bars, SEM.

225

226 Determining patch contributions to locomotion and place preference

227 Patch stimulation could modify responding by being inherently rewarding (White and
228 Hiroi, 1998). To explore this possibility, we next investigated the effects of patch stimulation on
229 reinforcement in a place preference task. To test the effects of optogenetic stimulation on
230 reinforcement, mice began a two-day real-time place preference task in a 2-chamber apparatus.
231 Entry to a randomly selected side resulted in laser stimulation (5 sec ON and 5 sec OFF, cycled),
232 which ended upon entrance to the opposite chamber. The stimulation side was counterbalanced
233 across 2 days and preference was averaged between days (see Methods). Patch activation did not
234 drive differences in time spent on the stimulation side relative to YFP controls (unpaired t-test, t_{27}
235 = 1.143, $p = 0.2631$; Figure 5A). These results suggest that optogenetic stimulation of patches is
236 not inherently reinforcing in this place preference task.

237



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240 **Figure 5. Effects of optogenetic stimulation of patches on reinforcement and locomotion.** A. Time spent on
241 stimulated side of a two-chamber place preference apparatus. Time is averaged between two days, and stimulated side

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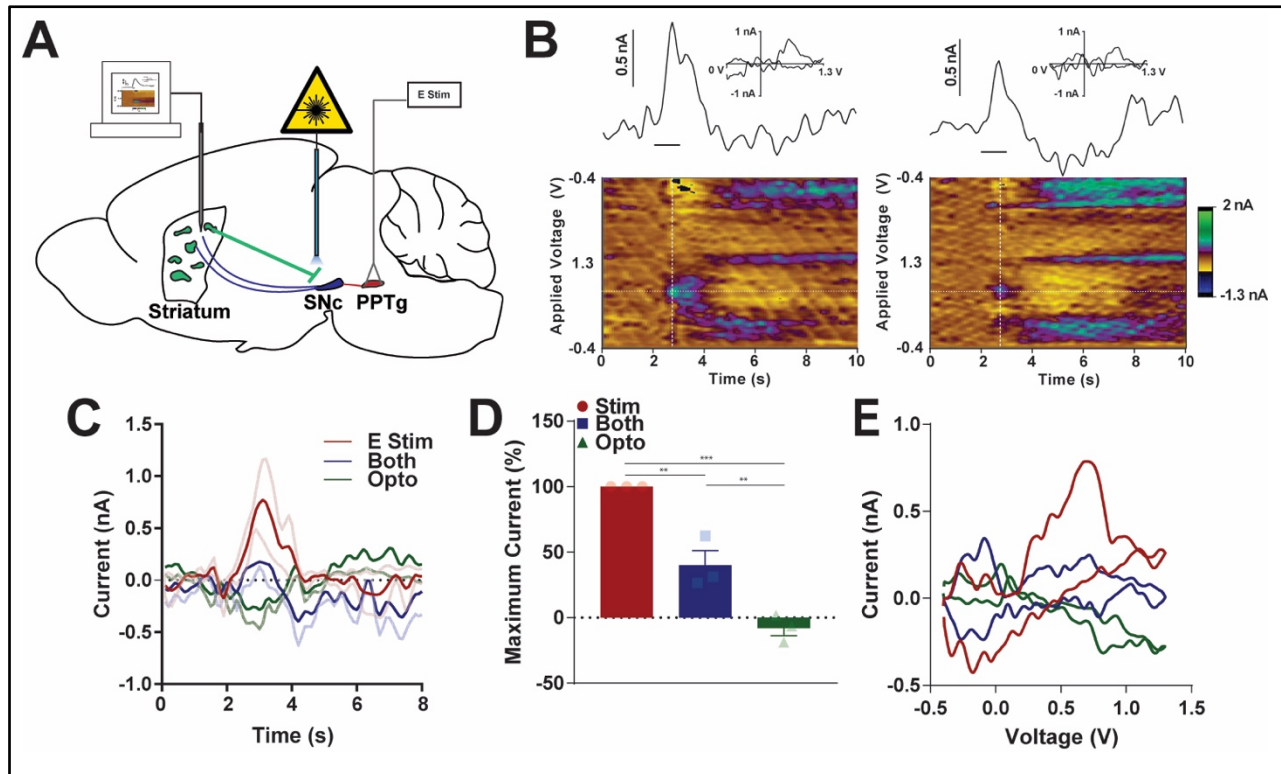
242 was counter balanced between days (see Methods). B. Average velocity in the open field for YFP and ChR2 mice. C.
243 Change in velocity following laser onset in open field. D-E. Average baseline normalized velocity before and after
244 laser onset (5 Hz, 3 sec; denoted by thick black line) for YFP (D) and ChR2 (E) mice. * $P < 0.05$; error bars, SEM.

245
246 Due to a predominant D1 dopamine receptor makeup (Smith et al., 2016), patch stimulation
247 may alter behavior by invigorating movement (Kravitz et al., 2010). To explore this possibility,
248 mice were placed in a custom open field chamber to assess the effects of patch stimulation on
249 locomotion, and laser stimulation occurred every 60 sec while the location of the mouse was
250 tracked (see Methods). We first assessed if intermittent patch stimulation elevated average
251 locomotion throughout the task, and found no difference in movement speed between ChR2 and
252 YFP mice (unpaired t-test, $t_{17} = 0.1127$, $p = 0.9116$; Figure 5B). However, onset of laser
253 stimulation significantly increased locomotion in ChR2 mice relative to controls (unpaired t-test,
254 $t_{17} = 2.708$, $p = 0.0149$; Figure 5C). On a finer timescale, YFP controls showed modest responses
255 to laser stimulation (Figure 5D). On the other hand, ChR2 mice demonstrated robust initial
256 increases following laser onset, which plateaued until cessation of stimulation, followed by a short
257 reduction in movement (Figure 5E). Together, these results show that patch activation can acutely
258 drive locomotion without being intrinsically reinforcing.

259 260 **Characterization of patch and dopamine interactions *in vivo***

261 As striatal patches are a unique striatal output population projecting to SNc (Crittenden et
262 al., 2016; Davis et al., 2018; Evans et al., 2020), optogenetic stimulation of patches may alter
263 responding by modulating dopamine release. We aimed to investigate this possibility by eliciting
264 phasic increases in striatal dopamine with electrical stimulation of the pedunculopontine tegmental
265 nucleus (PPTg; Forster and Blaha, 2003; Zweifel et al., 2009) with and without simultaneous
266 optogenetic activation of patch projections. We first injected a group of Sepw1-NP67 mice with
267 AAV encoding Cre-dependent ChR2. Three weeks later, mice were anesthetized and a glass-sealed
268 carbon-fiber microelectrode capable of detecting real-time changes in dopamine with fast-scan
269 cyclic voltammetry was lowered into the dorsal striatum. Following this, a bipolar stimulating
270 electrode was placed in the PPTg. Once robust dopamine was detected, a fiber optic was lowered
271 into the SNc targeting patch terminals (see Figure 6A for experimental design).

272 Stimulation of PPTg resulted in increases in striatal dopamine that mimicked naturally
273 occurring phasic increases in dopamine (Howard et al., 2013; Robinson et al., 2002; Figure 6B,
274 left). When PPTg stimulation occurred simultaneously with optogenetic patch activation, phasic
275 dopamine responses were present, but reduced in amplitude (Figure 6B, right). On average, PPTg
276 stimulation alone resulted in larger responses relative to simultaneous electrical and optogenetic
277 stimulation. On the other hand, optogenetic patch activation alone caused a small decrease in
278 detected current (Figure 6C; Supporting Figure 6A). When recordings were normalized to average
279 PPTg stimulation recording amplitude to account for baseline differences in release between
280 subjects, PPTg stimulation drove a larger dopamine response than simultaneous PPTg and patch
281 activation, which was significantly higher than optogenetic activation alone (One-way ANOVA,
282 $F_{(2,6)} = 53.97$, $p < 0.0001$; *post hoc* Tukey multiple comparison test all $p < 0.01$; Figure 6D).



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284

285 **Figure 6. Characterizing optogenetic patch stimulation on striatal dopamine release.** A. Experimental design.
286 Sepw1 NP67 mice were injected with AAV5 driving Cre-dependent expression of ChR2-eYFP. Fast-scan cyclic
287 voltammetry was used to monitor real-time changes in dopamine levels in striatum. An electrical stimulating
288 electrode was placed in pedunculopontine nucleus (PPTg) to drive increases in dopamine in dorsal striatum. A fiber
289 optic was placed above patch terminals in substantia nigra pars compacta (SNc). Electrical or optogenetic or both
290 stimulation types were randomly selected and delivered a minimum of three times each (see Methods). B. (left)
291 Representative recording of electrical stimulation of PPTg. Here, a line shows recorded current relative to
292 stimulation delivery (straight line below current trace) above a pseudo-color plot. The color plot shows current
293 collected (in color) at each waveform scan (y-axis) and across time (x-axis). INSET: a “cyclic voltammogram”
294 collected at the vertical white dotted line on the pseudo-color plot suggesting that dopamine is the predominate
295 analyte being monitored. (right) Same as left, but optogenetic stimulation of patch terminals occurs simultaneously
296 with PPTg electrical stimulation. C. Average responses across replicates for each of the three stimulation types.
297 PPTg stimulation only denoted as E Stim, optogenetic stimulation of patch terminals as Opto. D. Maximum
298 recorded current during stimulation normalized to electrical PPTg stimulation. E. Average cyclic voltammogram
299 across replicates for each stimulation type. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; error bars, SEM.

300

301 Inspection of average cyclic voltammograms further suggests the analyte detected following PPTg
302 stimulation is dopamine, and the peak oxidation is reduced following simultaneous optogenetic
303 and electrical stimulation (Figure 6E; average current and cyclic voltammograms for each
304 experimental replicate shown in Supporting Figure 6B). These results suggest that patch
305 projections to dopamine neurons are capable of suppressing dopamine release in the dorsal
306 striatum, which may contribute to the effects noted in the behavioral tasks.

307

308

309 **Discussion**

310 The striatum is a key locus in the transition from flexible to habitual strategies, but less is known
311 about how particular striatal subcircuits contribute to this phenomenon. Previous studies have
312 implicated striatal patches, or striosomes, in this process (Canales and Graybiel, 2000; Murray et
313 al., 2015, 2014), and more recent work has demonstrated that intact patches are necessary for
314 normal habit formation (Jenrette et al., 2019; Nadel et al., 2020). The current work further
315 addresses the role of patches in habit through use of optogenetics to modulate patch neuron activity
316 in a temporally-precise manner during habit formation. Patch stimulation enhanced behavioral
317 variability and invigorated responding across training. Additionally, optogenetic stimulation of
318 patch activity during learning suppressed the rate of lever pressing and head entry to the reward
319 port following reward devaluation, suggesting impaired habit formation. Next, we developed a
320 novel probe trial aimed at determining how mice respond following stimulation of patches
321 following reward devaluation. In these so-called ‘laser probe’ trials, stimulation of patches acutely
322 suppressed lever pressing and augmented head entry to the food port, suggesting acute patch
323 activation can drive habitual reward seeking. These effects were likely not attributable to directly
324 reinforcing effects of patch stimulation as assessed in a place preference task, though stimulation
325 of patches was sufficient to augment locomotion in an open field. Finally, we demonstrated that
326 optogenetic stimulation of patch terminals is sufficient to suppress dopamine release driven by
327 electrical stimulation of excitatory inputs to dopamine neurons. Together, these results suggest that
328 patches are a key site of habit formation and that patch activation can modify habitual responding,
329 potentially through regulation of striatal dopamine levels.

330

331 **Patches as a locus of habitual behavior**

332 The finding that patch manipulation impairs habitual responding is supported by previous studies
333 using Cre-dependent caspase lesions (Nadel et al., 2020) or a conjugated cytotoxin that selectively
334 ablates μ -opioid receptor expressing neurons (Jenrette et al., 2019), both finding impairments in
335 habitual responding. These patch manipulations, and the optogenetic approach utilized here, target
336 the central striatum, likely affecting patches in both medial and lateral striatum. A now well-
337 supported model proposes that the medial striatum encodes goal-oriented behavior, while the
338 lateral striatum encodes habitual responding (Yin and Knowlton, 2006), a distinction which may
339 also apply to dopamine neurons (Faure et al., 2005; Lerner et al., 2015). Patches span the
340 medial/lateral spectrum of the striatum, and studies manipulating patches specifically within
341 medial or lateral striatum should be pursued to determine if there is a functional divide within
342 medial and lateral patches.

343 One puzzling aspect of the current work is how activation of patches through the use of
344 optogenetics could impair habit formation, when lesioning patches has a similar effect (Jenrette et
345 al., 2019; Nadel et al., 2020). We propose two ideas to explain this paradoxical finding. First,
346 optogenetic stimulation may enhance ongoing patch activity, but could impair the timing of
347 spiking relative to afferent activation during learning, thus disrupting plasticity during the
348 transition to habitual responding. Striatal neurons have been shown to be highly sensitive to spike-

349 timing of corticostriatal inputs, and modifying afferent and spiny projection neuron spike timing
350 by milliseconds can reverse the valence of plasticity in these cells (Fino and Venance, 2010). We
351 chose to deliver optogenetic stimulation during reward retrieval based on previous studies showing
352 activity increases in patches to rewards or cues predicting rewards (Bloem et al., 2017; Yoshizawa
353 et al., 2018). However, direct electrophysiological or optical recordings of patches would be
354 required to determine patch activity in this variable interval schedule. Alternatively, optogenetic
355 activation of patches may drive rebound inhibition, which may lead to impairments in habit
356 formation by suppressing patch activity following cessation of laser firing. Rebound excitation and
357 inhibition have been well characterized during inhibition or excitation of neuronal circuits with
358 optogenetics (Häusser, 2014). Indeed, we note potential behavioral evidence of this phenomenon
359 in the current work: patch activation in open field drives robust increase in locomotion, followed
360 by a brief inhibition of movement where locomotor behaviors fall below baseline (Figure 5E).
361 Future physiological studies of patch function should explore how patch activity is modified during
362 habit learning.

363

364 **Patch manipulation decreases responding in devaluation probe trials**

365 Reduced responding during devaluation probes could be partially explained by an elevated
366 response rate during stimulated VI60 trials. That is, if laser stimulation invigorates responding,
367 normalizing responding to an elevated baseline may drive this effect. While this possibility cannot
368 be completely resolved, a lower number of total head entries, which are not normalized to baseline
369 (Figure 3C), argues against this being the only factor contributing to reduced responding following
370 devaluation. An additional limitation of the current study is the lack of a matrix specific
371 manipulation to determine specificity of this effect is to patches. Indeed, matrix lesions have been
372 shown to impair fine motor coordination (Lopez-Huerta et al., 2016), but the role of the matrix has
373 not been investigated in habit formation. However, this work suggests that specific manipulation
374 of patch neurons is sufficient to alter habit formation, adding to a growing body of literature
375 indicating patches are a key site in the transition to habitual responding (Canales and Graybiel,
376 2000; Jenrette et al., 2019; Murray et al., 2015, 2014; Nadel et al., 2020).

377 Importantly, the current work lacks a “non-devalued”, or “valuation” probe trial, which
378 controls for general satiety following free access to rewards (Balleine and Dickinson, 1998;
379 Colwill and Rescorla, 1985). Valuation probes often utilize free access to a reward that is different
380 from rewards earned during in training (eg. maltodextrin solution, homecage chow; Nelson and
381 Killcross, 2006; Shillinglaw et al., 2014; Yu et al., 2009) and several studies have described the
382 degree of habit as a ratio of responding in devaluation vs. valuation probe trials (Gremel and Costa,
383 2013; O’Hare et al., 2017, 2016). Valuation probes were omitted in the current study due to our
384 previous finding that Sepw1-NP67 mice rapidly suppress responding across two days of probe
385 trials regardless of reward type, which obscured results of devaluation (Nadel et al., 2020). When
386 sucrose was weighed before and after free access in a subgroup of mice, we found no difference
387 in the weight of sucrose consumed (Figure S3A+B) suggesting similar satiety across animals.

388 Nevertheless, lack of a control to ensure reward-specific devaluation is a potential confound of the
389 current study.

390 Stimulation of patches across training reduced head entry to the food magazine in
391 devaluation trials. On the other hand, acute activation of patches following reward devaluation was
392 sufficient to drive head entry to the food magazine. This unexpected finding could suggest that
393 patch activation directly drives habitual reward seeking. Previous studies have utilized head entry
394 as a metric for habitual responding and reward devaluation has been shown to reduce head entry
395 in goal-directed animals (DePoy et al., 2016; Morrison et al., 2015; Rode et al., 2020). Discrete
396 head entry and lever pressing events could be ‘chunked’ into larger learned action sequences that
397 are reinforced across habit formation (Dezfouli and Balleine, 2013). Striatal patches may serve as
398 a neural substrate of this hierarchical reinforcement, as both press and entry rate are reduced in
399 devaluation probes following patch manipulation. Because striatum is necessary for expression of
400 learned action sequences (Berridge and Whishaw, 1992; Yin, 2010), and as striatal activity
401 encodes action chunking (Jin et al., 2014; Jin and Costa, 2010) with differential contribution of
402 direct and indirect pathways (Geddes et al., 2018), future studies should explore the contribution
403 of patch/matrix subcircuits on sequence learning.

404

405 **Patches and behavioral variability**

406 During training, optogenetic stimulation of patches resulted in lower autocorrelation
407 coefficients (Figure 2E+I), suggesting impaired day-to-day consistency in responding. Further,
408 correlation of responding during retraining and omission probes were disrupted in ChR2 mice
409 (Supporting Figure 1G-H) which may reflect enhanced behavioral variability across days. These
410 findings are supported by our previous study, which found that Cre-dependent lesions in Sepw1-
411 NP67 mice similarly disrupted autocorrelations and increased behavioral variability (Nadel et al.,
412 2020). These studies together suggest that patches may support habit formation by facilitating
413 crystallization of action patterns. Indeed, generalized lesions of the dorsal striatum tend to increase
414 behavioral variability in foraging tasks (Charnov, 1976; Compton, 2004). In support of this notion,
415 mice in “laser probe” trials show a correlation between response rates in retraining and following
416 devaluation (Supporting Figure 4 K-L). It is possible that patch neurons may play a general role in
417 reducing behavioral variability across learning, though more studies are required to directly test
418 this idea.

419

420 **Patches and behavioral invigoration**

421 Additionally, in training, we found that optogenetic stimulation of patches tended to
422 enhance ongoing behaviors. ChR2 mice showed slightly increased press and entry rates during
423 VI60 (Figure 2D+H), increased locomotion in open field (Figure 5C-E), and drove increased entry
424 into the food port during laser probes (Figure 4D+F). This may be due to an enriched population
425 of direct-pathway, D1 dopamine receptor-expressing neurons in patches (Miyamoto et al., 2018;
426 Smith et al., 2016). Indeed, optogenetic stimulation of D1 populations enhances movement
427 (Kravitz et al., 2010) through inhibition of basal ganglia output nuclei (Freeze et al., 2013).

428 However, a recent study suggests that striosomes can be further subdivided into functionally
429 distinct populations, both of which predominantly express D1 receptors. Contrary to the current
430 work, optogenetic stimulation of Teashirt family zinc finger 1 (Tshz1) expressing neurons in
431 striosomes drives aversion and suppression of movement. On the other hand, optogenetic
432 activation of prodynorphin expressing neurons, which are also enriched in patches drives
433 reinforcement and activation of movement (Xiao et al., 2020). Based on this, it is possible that our
434 Sepw1 NP67 line overlaps more closely with prodynorphin-Cre mice, which is supported by
435 previous work (Smith et al., 2016). Future studies will undoubtedly move toward further
436 subdividing diverse neuron populations in patches and matrix to determine their role in behavioral
437 regulation.

438

439 **Patch-dopamine interactions**

440 This work provides new insight into the relationship between striatal patches and dopamine
441 release, demonstrating that optogenetic stimulation of patch projections suppresses dopamine
442 release in the dorsal striatum (Figure 6). Previous studies have supported this notion demonstrating
443 anatomical (Crittenden et al., 2016; Fujiyama et al., 2011; Gerfen, 1985; Watabe-Uchida et al.,
444 2012) and functional connectivity (Evans et al., 2020; McGregor et al., 2019). Patches could
445 therefore regulate habitual behavior by sculpting dopamine release across learning. Indeed,
446 dopamine responses transition from ventromedial to dorsolateral striatum as behaviors become
447 well learned (Willuhn et al., 2012), and patches could be involved in gating of dopamine release
448 early in learning. Moreover, patches could provide feed-forward inhibition to dopamine neurons,
449 which may facilitate the activity shift from reward to cue during Pavlovian conditioning (Schultz,
450 1998), and which could drive negative dopamine responses during reward omission (Watabe-
451 Uchida et al., 2017). Very recent work suggests that patch-dopamine interactions are also
452 reciprocal, as dopamine differentially modulates patch neuron activity relative to matrix neurons
453 (Prager and Plotkin, 2018). Based on this proposed role of patches regulating dopamine release
454 and habitual behaviors, it will be of great interest to explore how patches contribute to pathological
455 compulsive states including drug addiction and Obsessive Compulsive Disorder.

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468 **Materials and methods**

469 **Key Resources Table**

strain, strain background (<i>Mus musculus</i>)	Sepw1-NP7-Cre	Charles Gerfen (National Institutes of Health) and Nathaniel Heintz (Rockefeller University) (Gerfen <i>et al.</i> 2013)	RRID:SCR_011431	
strain, strain background (<i>Adeno-associated virus</i>)	AAV-EF1a-DIO-hChR2(H134R)-EYFP-WPRE-pA	UNC Viral Vector Core	RRID:SCR_002448	4x10 ¹² particles per ml
strain, strain background (<i>Adeno-associated virus</i>)	AAV-EF1a-DIO-EYFP	UNC Viral Vector Core	RRID:SCR_002448	3.5x10 ¹² particles per ml
antibody	anti-MOR antibody	Immunostar (24216)	RRID:AB_572251	1::1000
antibody	2° antibody	Jackson Laboratory (711-165-152)	RRID:AB_2307443	1::250

470

471 **Animals**

472 All experiments were in accordance with protocols approved by the Oberlin College Institutional
473 Animal Care and Use Committee. Mice were maintained on a 12 hr/12 hr light/dark cycle and
474 unless otherwise noted, were provided *ad libitum* access to water and food. Experiments were
475 carried out during the light cycle using 41 heterozygous Sepw1-Cre^{+/-} mice ranging from 2 to 6
476 months of age, which were generously provided by Charles Gerfen (National Institutes of Health)
477 and Nathaniel Heintz (Rockefeller University). These mice preferentially express Cre-
478 recombinase in striatal patches (Gerfen *et al.*, 2013; Smith *et al.*, 2016, Figure 1C+D).

479

480 **Reagents**

481 Isoflurane anesthesia was obtained from Patterson Veterinary (Greeley, CO, USA). Sterile and
482 filtered phosphate buffered saline (PBS, 1X) was obtained from GE Life Sciences (Pittsburgh, PA,
483 USA). Unless otherwise noted, all other reagents were obtained through VWR (Radnor, PA, USA).

484

485 **Stereotaxic Surgery and Viral Injections**

486 Sepw1-NP67 mice were anaesthetized with isoflurane (4% at 2 L/sec O₂ for induction, 0.5-1.5%
487 at 0.5 L/sec O₂ afterward) and then placed in a stereotaxic frame (David Kopf Instruments,
488 Tajunga, CA, USA). The scalp was sterilized with povidone iodine and an incision was made in
489 the scalp. For optogenetic experiments, the skull was scored with Optibond (Patterson Dental).
490 Holes were then drilled bilaterally above the dorsal striatum (+0.9 AP, 1.8 ML, -2.5 DV) and 500
491 nL of an AAV encoding channelrhodopsin (ChR2) (AAV-EF1-DIO-hChR2(H134R)-EYFP-

492 WPRE-pA, UNC Viral Vector Core) was injected. Control mice were injected with an AAV
493 encoding YFP (AAV-EF1a-DIO-EYFP, UNC Viral Vector Core). For all injections, a 5 μ L
494 syringe needle (Hamilton) was lowered to the DV coordinate over 2 minutes and held in place for
495 1 min before the start of injection. The injection speed was 100 nL/min, and the needle was left
496 undisturbed in the brain for 5 minutes after the completion of virus delivery, after which the needle
497 was removed over the course of 5 minutes. Fiber optics were then inserted bilaterally targeting one
498 of three sites: cell bodies of patch neurons in the striatum (+0.9 AP, 1.8 ML, -2.3 DV), patch
499 terminals at dopamine neurons of the substantia nigra pars compacta (-3.2 AP, 1.5 ML, -3.6 DV),
500 or over patch terminals in the entopeduncular nucleus EP (-1.1 AP, 2.1 ML, -4.0 DV; Figure
501 1C+D), and secured to the skull with dental cement (Patterson Dental). Control mice expressing
502 YFP had fiber optics implanted targeting one of these three sites selected randomly. Carprofen (5
503 mg/kg, subcutaneous) was used for postoperative analgesia. A subset of mice were injected with
504 AAV encoding ChR2 but did not receive fiber optic implants. These mice instead received sterile
505 sutures to close the incision site (see Fast-Scan Cyclic Voltammetry below). All mice were given
506 3-4 weeks to allow for viral expression and to recover before behavioral training started.

507

508 **Variable Interval Training**

509 Mice were trained on a variable interval schedule to induce habitual responding (Rossi and Yin,
510 2012, see Figure 2A for schematic of entire behavioral training protocol). Throughout training,
511 mice were food deprived and kept at ~85% initial weight by daily feeding of 1.5-2.5g of homecage
512 chow daily after training. All instrumental learning experiments were performed in standard
513 operant chambers (Med Associates). Each chamber had a retractable lever on either side of a
514 reward bowl, which was linked to a sucrose-filled syringe that delivered liquid reward (10%
515 sucrose solution, 20 μ l) and a house light on the opposite side of the chamber. Briefly, mice first
516 underwent four days of continuous reinforcement (CRF, one lever press yields one reward) to
517 establish the association between lever press and reward. At the start of the session, the house light
518 was illuminated, and one lever was inserted into the chamber. After 60 min or 50 rewards, the light
519 was shut off, the lever was retracted, and the session ended. On the final day of CRF training, mice
520 were briefly anesthetized with isoflourane (4%, 2 l/min O₂) and were connected to fiber optic
521 leads to habituate mice to the optogenetic apparatus. Mice that failed to reach criteria within four
522 days were given an additional 1-2 days of CRF training. Subsequent behavioral trials began with
523 acute anesthetization with isoflourane and connection to fiber optic leads prior to training.
524 Following CRF training, mice experienced three days of a variable-interval (VI) 30 task, in which
525 they were rewarded on average 30 seconds (15-45 second range) contingent on lever pressing,
526 followed by 8 days of VI60 training (rewarded every 60 seconds on average, ranging from 30 to
527 90 seconds, with each possible interval separated by 6 sec) (Nadel et al., 2020). VI sessions ended
528 after 60 min or when 50 rewards had been earned. To assess the contribution of patches to habit
529 formation, mice received optogenetic stimulation (5 mW, 5 Hz, 190 ms pulse width, 3 sec duration,
530 see below) of patch neurons or terminals during the first headentry following each reward delivery
531 in all VI60 trials. Patch activity is linked to reward-predicting cues or during reward consumption

532 (Bloem et al., 2017; Yoshizawa et al., 2018), thus this stimulation timing was selected to modulate
533 ongoing activity in patch neurons.

534

535 **Fiber Optic Implants**

536 Fiber optic implants were custom fabricated and were comprised of 0.39 NA, 200 μ m core
537 Multimode Optical Fiber (ThorLabs) inserted into a multimode ceramic zirconia ferrules (1.25mm
538 OD, 230um ID; SENKO). The fiber optic was affixed in the ferrule with two-part epoxy (353ND;
539 Precision Fiber Products). Each end of the fiber optic was polished using fiber optic sandpaper
540 (ThorLabs) and functionality was tested ensuring minimal loss of light power and even output
541 prior to implantation.

542

543 **Laser Stimulation**

544 Mice received blue laser stimulation (473 nm, 5 mW, 5 Hz, 190 ms pulse width, 3 sec duration)
545 from a diode-pumped single-state laser (Laserglow) which was connected via fiber optic (Doric
546 Lenses) to a commutator (1x2 Fiber-optic Rotary Joint) allowing for free rotation and splitting of
547 the beam (Doric Lenses). The commutator was connected to two fiber optic leads, which were
548 attached bilaterally to ferrules on fiber optic implants with a ceramic sleeve (Precision Fiber
549 Products). Laser output was calibrated to 5 mW from the end of fiber optic leads before training
550 each day using an optical power meter (ThorLabs). Laser parameters were the same for all
551 behavioral tasks (VI60, Laser Probes, Open Field) with the exception of Real-Time Place
552 Preference, where laser stimulation duration was cycled 5 sec ON, then 5 sec OFF (see below).

553

554 **Probe Tests**

555 Following 8 days of VI60 training, a reward devaluation test was conducted. Here, mice were
556 given free access to sucrose for one hour prior to testing. Mice were individually caged during this
557 access and all mice were observed to ensure they consumed sucrose. To quantify sucrose
558 consumption, a subgroup of mice had sucrose bottles weighed before and after free access. After
559 the pre-feeding session, mice were given a 5-min probe test in which the lever was extended and
560 presses were recorded, but no rewards were delivered. Reward devaluation is commonly used to
561 probe habitual responding, and mice that persist in lever pressing during devaluation probes are
562 considered more habitual (Adams and Dickinson, 1981; Gremel and Costa, 2013; O'Hare et al.,
563 2016). Following devaluation probes, mice experienced one day of VI60 training to reinstate
564 habitual responding. The following two days, mice were also tested on a 60 minute omission probe
565 test in which the action-outcome contingency was reversed. Here, mice had to refrain from
566 pressing the lever for 20 seconds to obtain a reward, and pressing the lever reset the timer (Nadel
567 et al., 2020). This probe was employed as a second metric of habitual responding, as habitually
568 responding mice are slower to reverse learned action-outcome contingencies (Yu et al., 2009).
569 Following two days of omission trials, mice were again retrained on a VI60 schedule to reinstate
570 lever pressing. The following day, mice underwent a "laser probe" trial. Here, mice again
571 underwent reward devaluation by gaining free access to sucrose for one hour (as described above).

572 Mice were then returned to operant chambers and the lever was extended and presses were
573 recorded, but no rewards were delivered. At variable intervals between 30-90 sec (6 sec between
574 each possible interval) laser stimulation was delivered to fiber optic implants (5 mW, 5 Hz, 190
575 ms pulse width, 3 sec duration), and laser probe trials lasted a total of 30 min. This probe was
576 conducted to determine the acute effects of patch stimulation on responding following reward
577 devaluation.

578

579 **Real-Time Place Preference**

580 Following operant conditioning tasks, mice were returned to *ad libitum* access to homecage chow.
581 At least 3 days later, fiber optic implants were again connected to fiber optic leads and mice were
582 placed in a 2-chamber place preference apparatus (Med Associates). Each chamber was 16.8 cm
583 L x 12.7 cm W x 12.7 H with opaque walls. Chambers were distinguishable based on different
584 flooring (grid vs bars) and different wall coloring (white vs black), and the orientation of the
585 chamber did not change across place preference trials. To allow fiber optic movement and prevent
586 mice from exiting the chamber, a custom, clear plexiglass wall extension (45.7 cm tall, 58.4 cm
587 total height) was placed on the walls above the behavioral apparatus and no lid was utilized. Mice
588 underwent two days of real-time place preference trials. Here, one chamber was randomly selected
589 to trigger laser stimulation when mice entered or remained in the ‘active’ chamber, and the active
590 chamber was counterbalanced across days. Location in the chambers was monitored by 12 evenly-
591 spaced infrared beam breaks located near the floor of the apparatus. At the first occurrence of a
592 beam break on the active side, laser stimulation was delivered to the fiber optic implants (5 mW,
593 5 Hz, 190 ms pulse width). As striatal stimulation can result in freezing depending on the neuronal
594 population activated (Kravitz et al., 2010), laser stimulation was cycled ON for 5 sec and OFF for
595 5 sec. This pattern of stimulation occurred until a beam break occurred in the inactive chamber,
596 when stimulation was halted until the next beam break in the active chamber. Time spent on either
597 side was compared and averaged across each day to account for inherent preferences for either
598 side. This task was performed to determine if optogenetic patch stimulation was inherently
599 reinforcing, as suggested by a previous electrical self-stimulation experiment (White and Hiroi,
600 1998).

601

602 **Open Field**

603 At least one day following RTCPP trials, fiber optic implants were again connected to fiber optic
604 leads and mice were placed in an open field apparatus (42 cm wide x 42 cm long x 30 cm tall) to
605 determine the effects of acute patch stimulation on locomotor activity. Every 60 sec laser
606 stimulation (5 mW, 5 Hz, 190 ms pulse width, 3 sec duration) was delivered to implants. Mouse
607 locomotion was monitored by a camera and analyzed online using Bonsai software (Open-Ephys).
608 Movement was detected using a contrast-based binary region analysis and extraction of location
609 in the video frame (Lopes 2015 Frontiers in Bioinformatics).

610

611 **Fast-Scan Cyclic Voltammetry**

612 To determine the impact of patch activation on striatal dopamine release, we utilized fast-scan
613 cyclic voltammetry (FSCV) to monitor real-time changes in striatal dopamine levels while
614 simultaneously activating patch terminals with optogenetics *in vivo*. Fast-scan cyclic voltammetry
615 was performed using custom glass-sealed, carbon-fiber microelectrodes (Cahill et al., 1996;
616 Howard et al., 2011). Recordings were made by applying a triangular waveform (0.4 to 1.3 V and
617 back, 400 V/s) every 100ms to the exposed tips of carbon-fiber microelectrodes. Voltammetry and
618 stimulus control was performed by a WaveNeuro potentiostat (Pine Research) and was computer-
619 controlled using HDCV software, which was generously provided by the Chemistry Department
620 at UNC (Bucher et al., 2013). A subset of Sepw1-NP67 mice were injected with AAV driving Cre-
621 dependent expression of Chr2 as described above. At least 3 weeks later, these mice were
622 anesthetized using urethane (1 g/kg i.p. delivered in 2 injections separated by ~20 min) and placed
623 in a stereotaxic apparatus. An incision was made in the scalp and holes were drilled above the
624 dorsal striatum (+0.8 AP, ±1.5 ML), SNc (-3.2 AP, ±1.5 ML), and pedunculopontine tegmental
625 nucleus (PPTg, -0.68 AP from lambda, ±0.7 ML). The PPTg sends excitatory projections to
626 dopamine neurons and was targeted with electrical stimulation to elicit dopamine release in the
627 striatum (Forster and Blaha, 2003; Zweifel et al., 2009). An Ag/AgCl reference electrode was
628 affixed in the superficial cortex. A carbon-fiber microelectrode was placed in the dorsal striatum
629 (-2.3 DV), and during implantation the carbon-fiber was cycled at 60 Hz to allow the electrode to
630 equilibrate and switched to 10 Hz ~20 min prior to data acquisition. A twisted bipolar stimulating
631 electrode (Plastics One, Roanoke, VA, USA) connected to a DS4 Biphasic Constant Current
632 Stimulus Isolator (Digitimer) was lowered in 0.1-mm increments starting at -1.5 DV into PPTg
633 until robust dopamine increases were detected in the dorsal striatum. Stimulus trains consisted of
634 60 biphasic pulses delivered at 60 Hz at a current of 400-600 µA and was synchronized with
635 recordings so that sampling and stimulation did not overlap. Stimulation intensity varied across
636 subjects to elicit robust dopamine release but was fixed at the beginning of data collection and did
637 not alter thereafter. Once stable dopamine release was detected, a fiber optic cable was inserted
638 above SNc (-2.0 DV) to target patch terminals and was incrementally lowered to optimize
639 placement (see Figure 6A for graphic of experimental design). Optogenetic stimulation consisted
640 of 1 sec pulses of blue laser light delivered at 5-10 mW. Three trial types were then conducted: 1.
641 Electrical stimulation of PPTg alone (“E stim” trials), 2. Optogenetic stimulation of patch terminals
642 in SNc (“Opto” trials), or 3. Simultaneous electrical stimulation of pedunculopontine tegmental
643 nucleus and optogenetic activation of patch terminals (“Both” trials). The order of trials was
644 selected randomly until one of each trial type had been collected, then this process was repeated a
645 minimum of 3 times. All recordings were separated by at least 3 minutes to avoid neurotransmitter
646 vesicle depletion.

647

648 **Histology and Microscopy**

649 At the cessation of all behavioral tests, mice were deeply anesthetized with isoflurane (4%, 2 l/min
650 O₂) and transcardially perfused with 0.9% saline and 4% paraformaldehyde (PFA). Brains were
651 removed and allowed to post-fix in 4% PFA at 4°C for at least 24 h. Brains were then transferred

652 to a 30% sucrose solution and returned to 4°C for at least 48 h. Brains were sectioned on a freezing
653 microtome into 20 µm sections. A subset of striatal sections from optogenetic experiments were
654 mounted and imaged to determine ChR2 expression. A separate set of sections from Sepw1-NP67
655 mice were washed 3X in Tris buffered saline (TBS) and blocked in 3% horse serum and 0.25%
656 Triton X-100 prior to antibody staining. Sections were then incubated in a 1:500 dilution of anti-
657 µ-opioid receptor polyclonal rabbit antibody (Immunostar, cat #24216) for 24–48 h at 4°C. A
658 separate set of tissue was procured from Sepw1-NP67 mice crossed to a Cre-dependent GFP-
659 reporter line to characterize Cre expression. This tissue was processed as described above, but was
660 incubated in a 1:500 dilution of anti-µ-opioid receptor polyclonal rabbit antibody (Immunostar, cat
661 #24216) and anti-GFP polyclonal guinea pig antibody (Synaptic Systems, cat#132–004) for 24–
662 48 h at 4°C. Tissue was visualized using a Leica DM4000B fluorescent microscope or a Zeiss LSM
663 880 confocal microscope.

664

665 **Data Analysis**

666 Mean and normalized press and head entry rates were compared across training and probe trials.
667 As press rates in mice with lesioned patches have been shown to be variable across training days
668 (Nadel), press and entry rates were normalized to average response rate across all VI60 trials to
669 compare to probe trials. Omission and laser probe press and entry rates were normalized to the
670 reinstatement VI60 training before each probe trial. We expected potentially opposing effects of
671 modulating differing terminal sites, but across VI60 training, devaluation probe trials, omission,
672 laser probe trials, open field, and place preference tasks we noted no statistical differences between
673 different fiber optic implantation sites in ChR2 groups (Supporting Figures 1-5), therefore, groups
674 were collapsed and comparisons were made between ChR2 mice and YFP controls. The ratio of
675 time spent in active:inactive chambers was averaged across two days of the place preference task
676 and then averaged across groups. Velocity in the open field was calibrated from megapixels/frame
677 to cm/sec using Matlab software MATLAB (R2018b, Mathworks). Press and entry rates were
678 calculated using Excel (Microsoft). Autocorrelations, cross-correlations, and real-time press and
679 entry rates in laser probes, were determined using custom scripts written in MATLAB (R2018b,
680 Mathworks). To control for individual differences in baseline responding and to determine laser-
681 induced changes in responding, laser probe press and entry rates were subtracted from baseline
682 responding 10 sec before laser onset before being averaged. To quantify responses in laser probe
683 trials, response rate was averaged across 1 sec just prior to and 5-8.5 sec following laser onset.
684 FSCV data was analyzed in HDCV (UNC Chemistry Department). Voltammetric current vs time
685 and current vs. voltage traces were collected and averaged for each trial type within experiments
686 (see above) before being averaged between subjects. Evoked amplitudes were normalized to
687 maximum current in PPTg stimulation only trials ('E stim' trials) to account for different
688 amplitudes of dopamine responses across subjects.

689

690 **Statistical Analysis**

691 Statistical analysis was performed by GraphPad Prism 7.04 (GraphPad) or Matlab (R2018b,
692 Mathworks). Press and entry rates during VI60 and omission probes were compared using Two-
693 Way Repeated Measures ANOVA with *post hoc* Sidak multiple comparison tests. Comparisons
694 between stimulation sites, evoked dopamine responses, and sucrose consumption between groups
695 were compared using One-Way ANOVA with *post hoc* Tukey's or Holm-Sidak multiple
696 comparison's tests. Press rates in VI60, VI30, and devaluation probes, as well as time on
697 stimulation side in place preference, baseline velocity, changes in velocity, and autocorrelations
698 were compared using unpaired student's t-tests. Changes in press and entry rates in laser probe
699 trials were compared using paired student's t-tests. Pearson's Correlation was utilized for all
700 correlations. Statistical outliers were determined using the ROUT (robust regression followed by
701 outlier identification) method (Q=0.5%) in GraphPad Prism 7.04 (GraphPad) and were removed
702 prior to statistical analyses. Finally, mice lacking ostensible viral expression in the striatum were
703 excluded prior to analysis. For all tests significance was defined as $p \leq 0.05$.

704

705 **Acknowledgements**

706 The authors would like to thank Drs. Charles Gerfen (National Institute of Mental Health) and
707 Nathaniel Heintz (The Rockefeller University) for generously providing Sepw1 NP67 mice. This
708 work was supported by NIH grant 1R15MH122729-01. J.A.N. was supported by the Nu Rho Psi
709 Undergraduate Research Grant and the Robert Rich Student Research Grant through Oberlin
710 College. Finally, the authors would also like to thank Lori Lindsay, Forrest Rose, Dorothy
711 Auble, Gigi Knight, Bill Mohler, Chris Mohler and Laurie Holcomb for research support.

712

713 **Competing Interests**

714 The authors declare that no competing interests exist.

715

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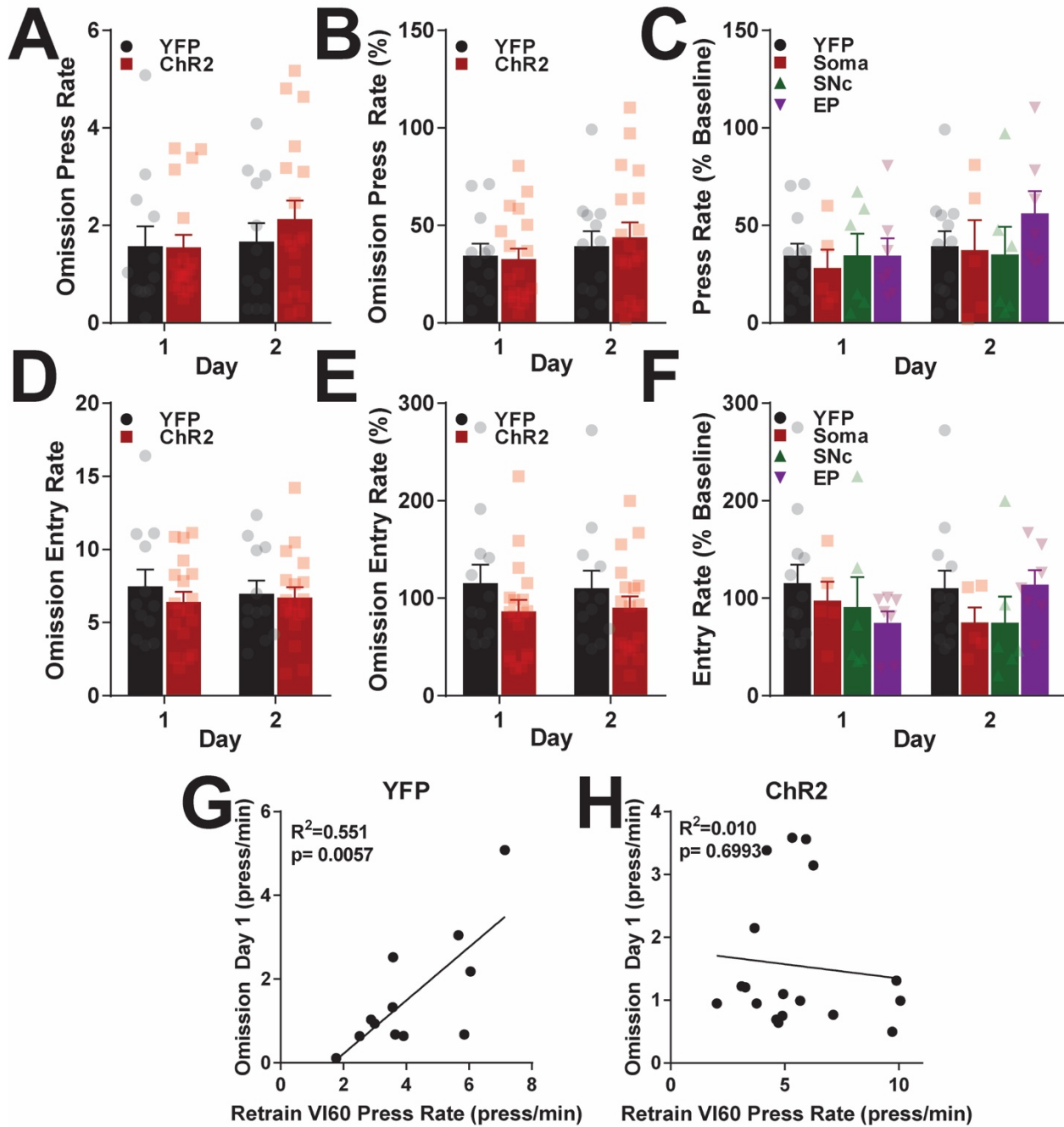
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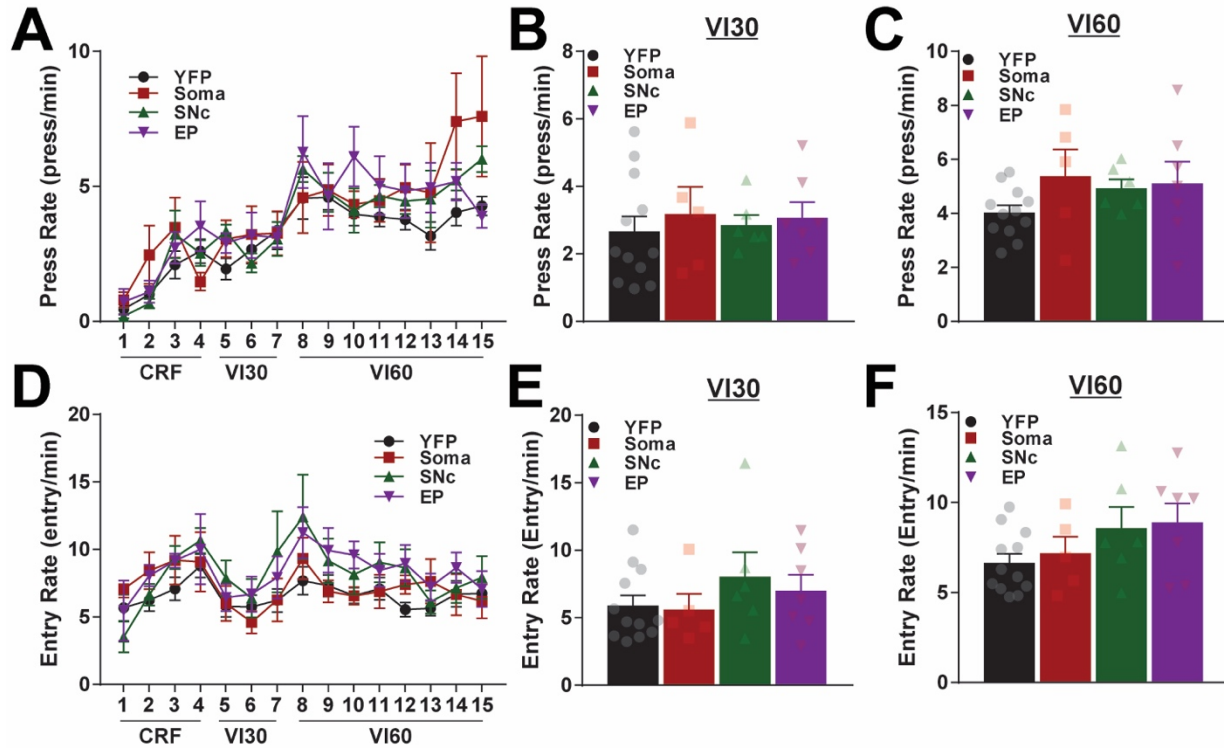
957 **Supporting Figures**



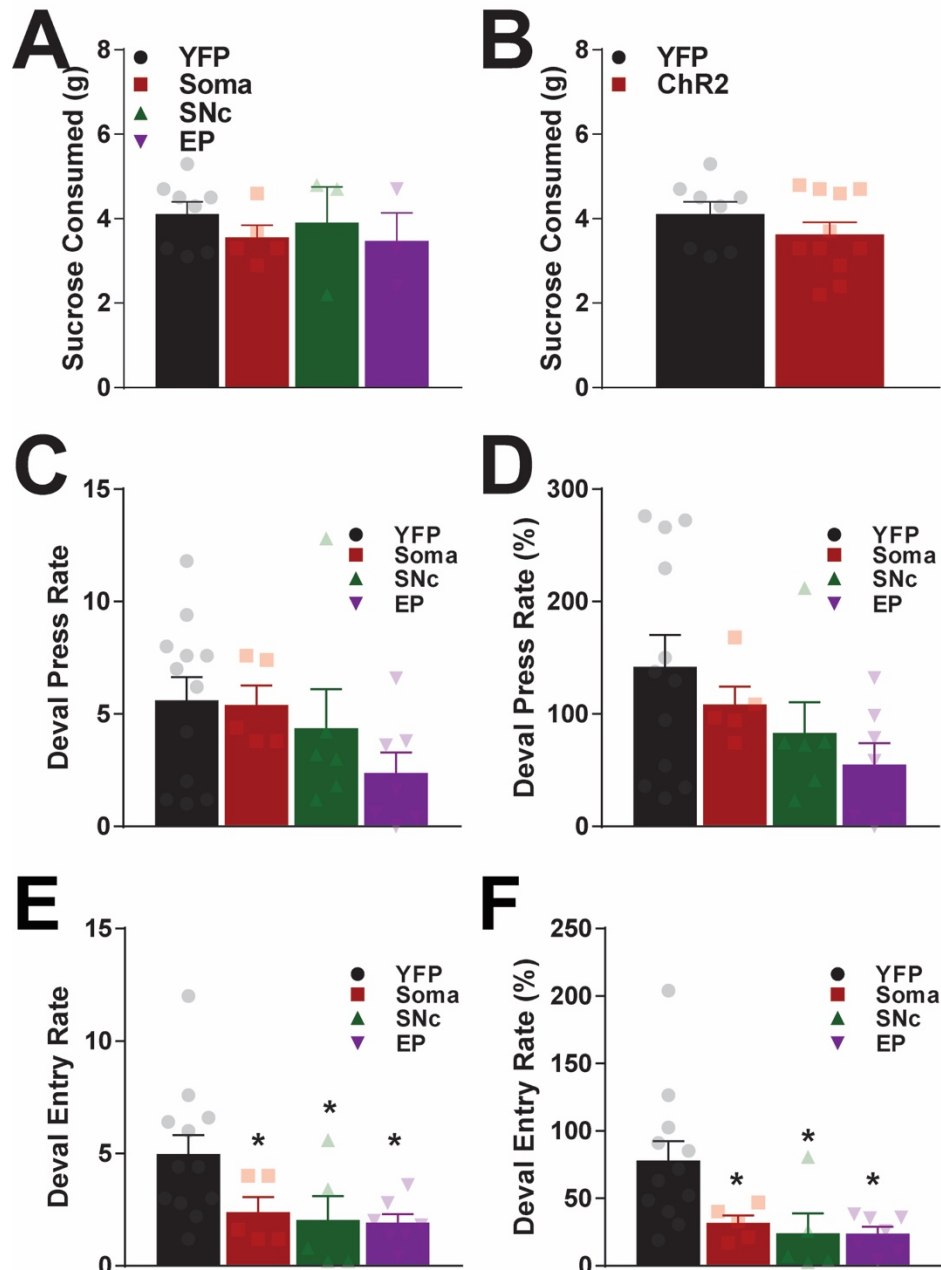
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960 **Supporting Figure 1. Effects of optogenetic patch stimulation during learning on omission.** A. Average press
961 rates during omission probes for YFP control and ChR2 mice across days (two-way repeated measures ANOVA, no
962 significant effects of time, group, or interaction). B. Average press rates normalized to responding during VI60
963 retraining during omission probes (two-way repeated measures ANOVA, no significant effects of time, group, or
964 interaction). C. Same data as B, but broken into fiber optic placement groups (two-way repeated measures ANOVA,
965 significant effect of time, $F_{(1,26)} = 4.56$, $p = 0.042$), no significant effect of group or interaction). D. Average head
966 entry rates during omission across days (two-way repeated measures ANOVA, no significant effects of time, group,
967 or interaction). E. Average entry rates normalized to baseline entry rates in VI60 retraining (two-way repeated

968 measures ANOVA, no significant effects of time, group, or interaction). F. Same as E, but broken into fiber optic
 969 placement groups (two-way repeated measures ANOVA, significant time x group interaction, $F_{(3,26)} = 3.87$, $p =$
 970 0.021 , no significant *post hoc* Tukey tests). G-H. Correlation of omission press rate on day 1 vs. VI60 retraining day
 971 immediately preceding omission for YFP (G) or ChR2 (H) mice. Data are mean \pm SEM.
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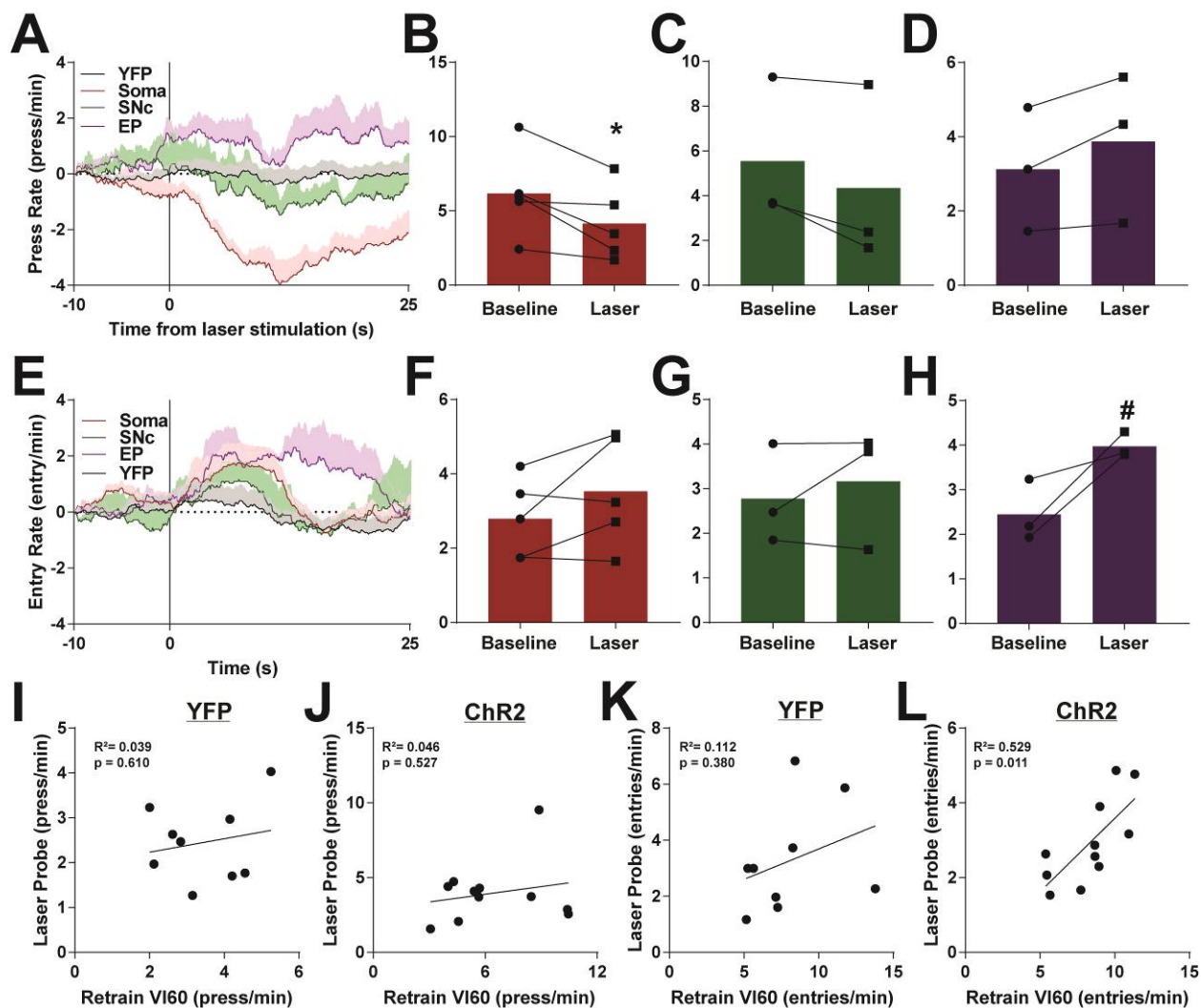


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 975 **Supporting Figure 2. Optogenetic patch stimulation during variable interval training by implantation site**
 976 **group.** A. Average press rates across continuous reinforcement (CRF), variable interval 30 (VI30), and variable
 977 interval 60 (VI60) training by day (two-way repeated measures ANOVA, significant effect of time, $F_{(14,364)} = 21.98$,
 978 $p < 0.0001$, no significant effects of group or interaction). B-C. Average press rates across all VI30 (B; one-way
 979 ANOVA, $F_{(3,26)} = 0.21$, $p = 0.89$) and VI60 (C; one-way ANOVA, $F_{(3,26)} = 1.34$, $p = 0.28$) days. D. Average head
 980 entry rates across training by day (two-way repeated measures ANOVA, significant effect of time, $F_{(14,364)} = 6.12$, p
 981 < 0.0001 , no significant effects of group or interaction). E-F. Average entry rate across all VI30 (E; one-way
 982 ANOVA, $F_{(3,26)} = 1.78$, $p = 0.18$) and VI60 (F; one-way ANOVA, $F_{(3,26)} = 0.79$, $p = 0.51$) days.
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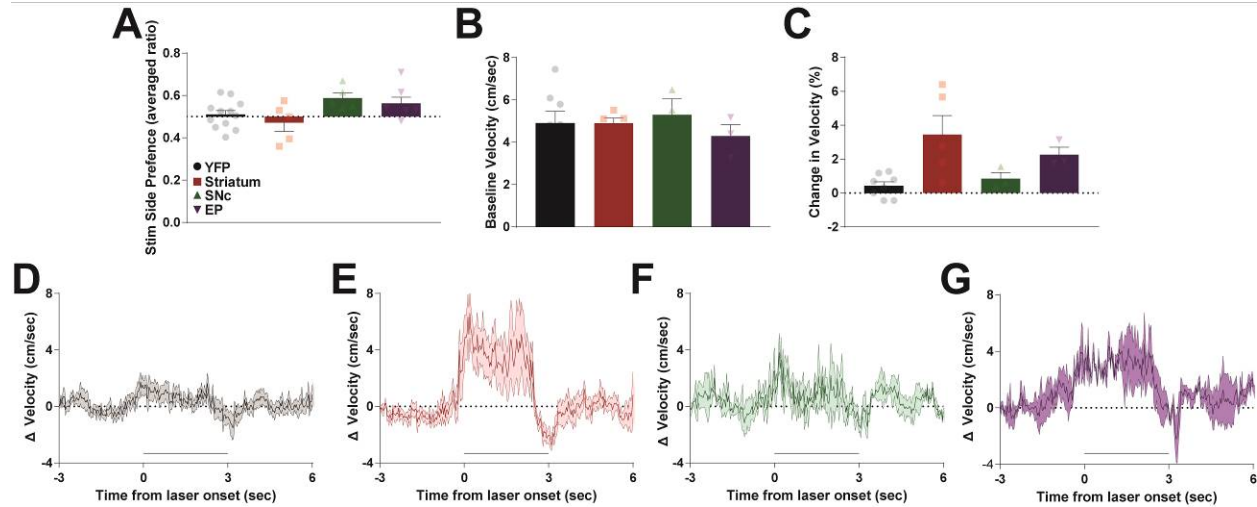
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Supporting Figure 3. Sucrose consumed during free access and press and entry rates in devaluation by implantation site group. A-B. Average sucrose consumed during 1h free access by implantation site group (A; one-way ANOVA, $F_{(3,15)} = 0.531$, $p = 0.67$) and collapsed into YFP/ChR2 groups (B; unpaired t-test, $t_{17} = 1.17$, $p = 0.26$). C-D. Average press rate (C; one-way ANOVA, $F_{(3,26)} = 1.53$, $p = 0.23$) and average press rates normalized to responding across VI60 training (D; one-way ANOVA, $F_{(3,26)} = 2.17$, $p = 0.12$). E-F. Average head entry rates (E; one-way ANOVA, $F_{(3,25)} = 3.626$, $p = 0.027$) and entry rates normalized to baseline VI60 responding (F; one-way ANOVA, $F_{(3,25)} = 4.644$, $p = 0.010$). Data are mean \pm SEM. *significant Holm-Sidak *post hoc* test



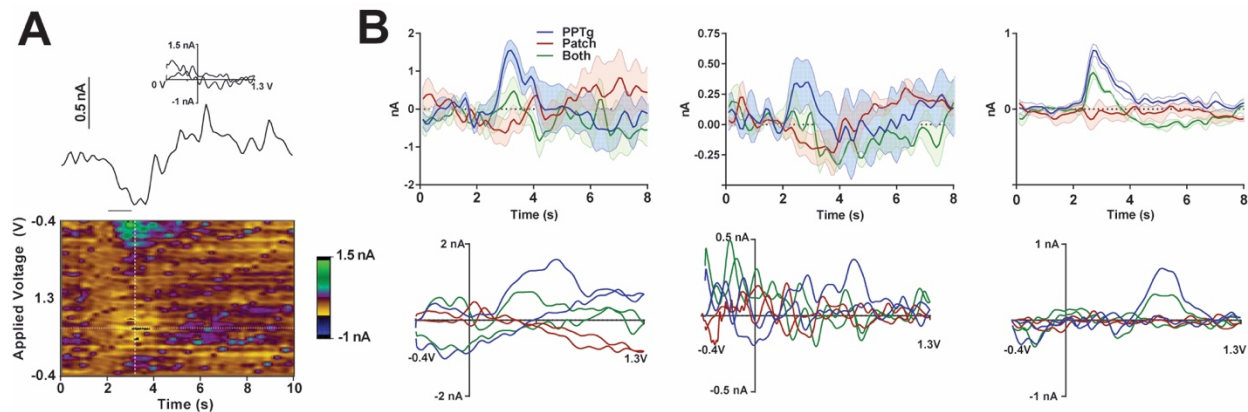
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998 **Supporting Figure 4. Effects of acute laser stimulation during laser probe trials by implantation site group.**
 999 A. Average lever press rate before and after laser onset (vertical line; 3 sec, 5 Hz stimulation). B-D. Average press
 1000 rates before and following laser onset for Soma (B; paired t-test, $t_4 = 3.10$, $p = 0.036$), SNc (C; paired t-test, $t_2 = 2.48$,
 1001 $p = 0.13$), and EP (D; paired t-test, $t_2 = 2.58$, $p = 0.12$) groups. E. Average head entry rates before and after laser
 1002 onset. F-H. Average entry rates before and after laser onset for Soma (F; paired t-test, $t_4 = 1.69$, $p = 0.17$), SNc (G;
 1003 paired t-test, $t_2 = 0.78$, $p = 0.51$), and EP (H; paired t-test, $t_2 = 3.24$, $p = 0.084$) groups. I-J. Correlation of lever press
 1004 rates during laser probe trials and VI60 retraining the day before for YFP (I) and ChR2 (J) mice. K-L. Correlation of
 1005 head entry rates during laser probe and VI60 retraining for YFP (K) and ChR2 (L) mice. Data are mean \pm SEM. # P
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Supporting Figure 5. Effects of optogenetic stimulation of patches on reinforcement and locomotion by implantation site groups. A. Time spent on stimulated side of a two-chamber place preference apparatus. Stimulation side was counterbalanced across two days and averaged between days (one-way ANOVA, $F_{(3,25)} = 3.023$, $p = 0.048$, no significant *post hoc* Holm-Sidak tests). B. Baseline velocity in open field (one-way ANOVA, $F_{(3,15)} = 0.33$, $p = 0.80$). C. Change in velocity following laser onset in open field (one-way ANOVA, $F_{(3,15)} = 5.22$, $p = 0.012$, no significant *post hoc* Holm-Sidak tests). D-E. Average baseline normalized velocity before and after laser onset (5 Hz, 3 sec; denoted by thick black line) for YFP (D), Soma (E), SNc (F), and EP (G) mice. Data are mean \pm SEM.



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Supporting Figure 6. Average data from each replicate in FSCV experiments. A. Representative decrease in current recorded during an "opto" trial (stimulation of patch terminals only). The line shows recorded current relative to stimulation delivery (straight line below current trace) above a pseudo-color plot. The color plot shows current collected (in color) at each waveform scan (y-axis) and across time (x-axis). INSET: a "cyclic voltammogram" collected at the vertical white dotted line on the pseudo-color plot. B. Average current (top) and cyclic voltammograms (bottom) recorded across three trials for each of the three stimulation conditions. Each current and cyclic voltammogram plot is from an individual FSCV experiment.