Lesion of striatal patches disrupts habitual behaviors and increases behavioral variability

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Abstract

Habits are automated behaviors that are insensitive to changes in behavioral outcomes. Habitual responding is thought to be mediated by striatum, with medial striatum guiding goal-directed action and lateral striatum promoting habits. However, interspersed throughout the striatum are neurochemically differing subcompartments known as patches, which are characterized by distinct molecular profiles relative to the surrounding matrix tissue. These structures have been thoroughly characterized neurochemically and anatomically, but little is known regarding their function. Patches have been shown to be selectively activated during inflexible motor stereotypies elicited by stimulants, suggesting that patches may subserve habitual behaviors. To explore this possibility, we utilized transgenic mice (Sepw1 NP67) expressing Cre recombinase in striatal patches to target these neurons for selective ablation with a virus driving Cre-dependent expression of caspase 3. Mice were then trained to press a lever for sucrose rewards on a variable interval schedule to elicit habitual responding. Mice were not impaired on the acquisition of this task, but lesioning striatal patches disrupted behavioral stability across training and lesioned mice utilized a more goal-directed behavioral strategy during training. Similarly, when mice were forced to omit responses to receive sucrose rewards, habitual responding was impaired in lesioned mice. To rule out effects of lesion on motor behaviors, mice were then tested for impairments in motor learning on a rotarod and locomotion in...
an open field. We found that patch lesions specifically impaired initial performance on the rotarod without modifying locomotor behaviors in open field. This work indicates that patches promote behavioral stability and habitual responding, adding to a growing literature implicating striatal patches in stimulus-response behaviors.

Introduction

Organisms must optimize behavioral strategies in order to be successful in their environments. However, various strategies exist for this purpose; optimization can be rapid and strongly dependent on outcomes or slow and resistant to change. Behaviors have therefore been divided into two main categories: goal-oriented and habitual behaviors [1]. Goal-directed, or action-outcome behaviors, are sensitive to the relationship between action and outcome and are thus highly flexible. In contrast, habitual, or stimulus-response strategies, are insensitive to changes in action-outcome relationships and lead to the continued use of behaviors that do not necessarily result in positive outcomes. While habitual strategies are evolutionarily advantageous by improving cognitive efficiency, maladaptive habit formation underlies pathological states including Obsessive Compulsive Disorder [2–4], drug addiction [5–7], and Tourette’s Syndrome [8]. These disorders are characterized by compulsive and maladaptive behaviors with common neuroanatomical alterations.

Habits have been studied in animal models by measuring perseverance of instrumental behaviors (e.g. lever pressing) following changes in action-outcome contingencies, often achieved through reward devaluation [9,10]. Using this approach, distinct neural circuits supporting goal-directed and habitual behaviors have been identified [2,11]. Impairment of the dorsomedial striatum, prelimbic cortex, or orbitofrontal cortex tend to disrupt goal-directed behaviors and animals become less sensitive to changes in outcomes [12–15]. In contrast, the lateral striatum functions as a key ‘habit center’, as lesions of this region promote flexibility [16]. This idea is consistent with human imaging studies, which find habitual behaviors correspond to overreliance the putamen, the primate homolog of the dorsolateral striatum [17,18]. A model has therefore been established suggesting that the dorsomedial striatum (DMS) and frontal cortical inputs facilitate goal-directed actions, while the dorsolateral striatum (DLS) promotes habitual behaviors [19], but see [20].

In addition to a medial-lateral divide, the dorsal striatum contains neurochemically distinct compartments: patches or striosomes make up approximately 15% of striatal volume and are surrounded by the remaining 85% of
the striatum, known as the matrix [21,22]. Patches were discovered nearly 50 years ago [23], and have since been
identified in human, monkey, cat, and rodent [24]. Despite decades of research into the neuroanatomy and
connectivity of striatal patches, their function remains poorly understood. Patches are heavily interconnected with
limbic circuits, and they provide the only direct inhibition to midbrain dopamine neurons from the striatum [25–27],
but see [28]. After repeated exposure, stimulant drugs of abuse drive expression of immediate early genes such as c-
fos selectively in patches, and this expression is predictive of motor stereotypies [21,29,30]. Similarly, lesions of
striatal patches reduce stimulant-induced motor stereotypies [31,32], suggesting patches may subserve compulsive
behaviors. Recent work has found that pharmacological ablation of μ-opioid containing neurons, which are enriched
in patches, disrupts habitual responding for sucrose rewards in rats [33]. In aggregate, these studies indicate a role
for patches in compulsive, habitual motor behaviors. To investigate patch involvement in habitual behaviors, we
utilized transgenic mice (Sepw1 NP67) which express Cre-recombinase in striatal patches [28,34]. We used a virus
driving Cre-dependent expression of caspase 3 to selectively ablate patch neurons before training mice on a variable
interval schedule of reinforcement, which has been previously used to establish habitual responding [9]. During
training, we noted significantly increased day-to-day variability in response rates in lesioned mice relative to
controls. Additionally, lesioned mice became more efficient across learning by suppressing unnecessary responses,
whereas control mice developed more stereotyped, inefficient patterns of responding. When mice were forced to
omit responses in order to earn rewards, lesioned mice had diminished response rates relative to control mice,
suggesting impaired habitual responding. Lesioned mice were also impaired on acquisition of motor learning as
assessed by performance on an accelerating, rotating balance rod (rotarod), though these mice show no generalized
locomotor impairments in open field. Together, this work supports the notion that patches subserve habitual
behaviors by promoting behavioral stability, an effect that cannot be solely attributed to deficits in motor control.

Materials and methods

Animals

All experiments were in accordance with protocols approved by the Oberlin College Institutional Animal Care and
Use Committee. Mice were maintained on a 12 hr/12 hr light/dark cycle and unless otherwise noted, were provided
ad libitum access to water and food. Experiments were carried out during the light cycle. Overall, 29 male and
female Sepw1-Cre/Rosa26-EGFP mice between 2 and 5 months of age were used in this study. Sepw1-Cre mice
were generously provided by Charles Gerfen (National Institutes of Health) and Nathanial Heintz (Rockefeller University). These mice show preferential Cre recombinase expression in striatal patches [28,34].

**Reagents**

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

**Viral injections**

To selectively ablate striatal patches, *Sepw1 NP67 X Rosa26-EGFP* mice were anaesthetized with isoflurane (4% at 2 L/sec O\(_2\) for induction, 0.5–1.5% at 0.5 L/sec O\(_2\) afterward), placed in a stereotactic frame (David Kopf Instruments, Tajunga, CA, USA), and were bilaterally injected with *AAV5-flex-taCasp3-TEVp* (UNC viral vector core). Cre-dependent expression of caspase 3 has been previously shown to drive apoptosis in neurons while limiting necrosis in surrounding tissue [35]. Briefly, two burr holes were drilled above dorsal striatum (+0.9 AP, ±1.8 ML, and −2.5 DV), and a 33-gauge needle was slowly lowered to the DV coordinate over 2 minutes and held in place for 1 min prior to injections. A 5 µl syringe (Hamilton) was used to inject 0.5 µl of virus over 5 min and the needle was left in place for 5 min following injections. The needle was then slowly retracted over 5 min. Mice were sutured and received Carprofen (5 mg/kg, s.c.) as postoperative analgesia. All mice were given 3 weeks to recover before behavioral training began. Control (non-lesion control) mice underwent an identical surgical procedure but received 0.5 µl of sterile, filtered phosphate-buffered saline (PBS).

**Variable Interval Training**

Mice were trained on a variable interval schedule to induce habitual responding ([9], see Fig 1D for experimental design). Throughout training, mice were food deprived and kept at 85% of initial weight by daily feeding of 1.5-2.5g of standard mouse chow. Operant conditioning was performed in standard operant chambers (Med Associates). Each chamber had two retractable levers on either side of a food magazine, where sucrose rewards were delivered (20% sucrose solution, 20 µl), and a house light on the opposite side of the chamber. Mice first underwent three days of continuous reinforcement training (FR1, one lever press yields one reward). At the start of the session, the house
light was illuminated, and the left lever was inserted into the chamber. After 60 min or 50 rewards, the light was
shut off, the lever was retracted, and the session ended. Animals that failed to obtain >10 rewards during FR1 were
given an extra day of FR1 training and were excluded if they did not reach this criterion. Next, mice were trained on
a variable-interval 30 task, in which they were rewarded on average 30 seconds (15-45 sec) contingent on lever
pressing. To determine how patch lesions modified habit formation across training, lesion and control mice were
divided into three groups experiencing either 3, 5, or 7 days of training on a VI60 schedule (rewarded every 60
seconds on average, ranging from 30 to 90 seconds). Variable interval sessions ended after 60 min or when 50
rewards had been earned.

Fig 1. Schematic of experimental design A. Schematic representation of injection sites in a coronal mouse
brain section. Sepw1-Cre mice express Cre recombinase in striatal patches (green). AAV5-AAV-flex-taCasp3-
TEVp (0.5 µl) or sterile PBS (control) was injected bilaterally into the dorsal striatum of Sepw1-Cre mice to
selectively lesion patches. B. Representative image of intact striatum of Sepw1-Cre X Rosa26-EGFP mice
displaying dense GFP expression in striatal patches. Dotted line denotes border of striatum and solid white line
denotes striatal patches. C. In lesioned mice, GFP + cells are greatly reduced and striatal patches are reduced in
number. D. Experimental design. Mice were trained to respond on a continuous reinforcement training (CRF) before
beginning variable interval 30 training (VI30). This was followed by variable interval 60 (VI60) training to establish
habitual responding. After training, mice experienced counterbalanced valuation/devaluation probes (Val, Deval,
respectively), followed by a day of reinstatement (VI60), and two days of omission (Omis). See Methods for details
of each behavioral schedule.

Probe trials

Following completion of VI training, a devaluation test was conducted over two days. Here, mice were allowed free
access to either chow (valuation) or sucrose solution (devaluation) for one hour. Immediately after, mice were given
a 5-min probe test in which the lever was extended and presses were recorded, but no rewards were delivered. The
order of the valued and devalued condition tests was randomized for each mouse. Mice that experienced 7 days of
VI60 training only underwent a single day of devaluation after finding a significant change in response rate across
probe days regardless of probe condition (see Results). One day after valuation and devaluation probe trials, mice
were reinstated on the VI60 task to reestablish response rates. The following two days, mice were tested with a 60-
minute omission test in which the action-outcome contingency was reversed such that mice were required to refrain
from pressing the lever for 20 seconds in order to receive rewards, and pressing the lever reset the counter. Omission
is a more robust means of extinguishing habitual responding [19], and was used to further probe habitual behaviors.
**Rotarod**

Deficits in operant behaviors could be due to changes in habit formation or due to generalized motor deficits. Therefore, following omission trials, mice were returned to *ad libitum* access to chow for at least one week prior to assessment of motor learning. We next sought to determine how lesions of striatal patches might affect motor learning using a rotarod (Ugo Basile). Mice were initially habituated to the rod by first walking for 5 min at a slow, constant rate of 4 rpm. Lesion or control animals were then trained with four trials per day for four days where the rotarod accelerated from 3-40 rotations per min over 360 sec [36]. Each trial ended when the mouse fell from the rod or after 360 sec had elapsed. A resting period of at least 15 min separated trials. Latency to fall was recorded and compared between lesion and control groups.

**Open field**

Following rotarod training, caspase-lesioned mice and controls were individually placed in a square activity chamber (42 cm wide x 42 cm long x 30 cm tall) and video-monitored from above for 30 minutes. After session completion, the distance moved, velocity, and rotation of each mouse was extracted from the video file using Ethovision (Noldus) and compared between control and lesion groups.

**Immunohistochemistry**

Following the completion of behavioral experiments, mice were anesthetized with isoflurane and transcardially perfused with 0.9% saline and 4% paraformaldehyde (PFA) using a peristaltic pump or manual injection. Brains were removed and allowed to post-fix in 4% PFA at 4°C for 24 h. Brains were then transferred to a 30% sucrose solution and returned to 4°C. Following sinking, brains were sectioned on a freezing microtome into 25 µm sections, which were stored in a cryoprotectant solution before being washed 3X in Tris buffered saline (TBS) and blocked in 3% horse serum and 0.25% Triton X-100. Sections were then incubated in a 1:500 dilution of anti-GFP polyclonal Guinea Pig antibody (Synaptic Systems, cat#132-004) for 24-48 h at 4°C on a shaker. Following incubation, sections were washed 2x15 minutes in TBS to remove excess primary antibody, then blocked for 30 minutes before incubating in Alexa Fluor® 488 AffiniPure Donkey Anti-Guinea Pig IgG (Jackson ImmunoResearch, cat#706-545-148, diluted 1:250) for 2 hours at room temperature. Tissue was then washed 3x15 min in TBS to reduce background staining. Slices were subsequently floated in 0.1M phosphate buffer (PB) and mounted on slides.
drying, sections were covered using mounting media (Aqua-Poly/Mount, Polysciences, 18606-20) with DAPI (Sigma-Aldrich D9542; 1:1000). Tissue was visualized using a Leica DM4000B fluorescent microscope.

Data and Statistical Analysis

Prior to comparison, devaluation probe rates for each mouse were normalized to valuation press rates (LPr; [37]) or average press rates across all VI60 trials. Reinstatement press rates were normalized to press rates during the final day of VI60. Omission press rates were normalized to press rate during the reinstatement day following devaluation probes. Autocorrelation (lag 1) of press rates across VI60 training and cross-correlation were determined using MATLAB (R2018b, Mathworks). We intended to investigate the effects of patch lesions across different VI60 training durations (3, 5, or 7 days), but found no effect of training days across multiple task metrics, including press rates on the final day of VI60 training, and normalized response rates during valuation/devaluation probes, reinstatement day, nor omission days (p > 0.05, data not shown). Therefore, we collapsed these three groups for subsequent analysis. However, due fewer training days in the 3-day group, variability and behavioral strategy analysis was reserved for mice that received 5 or 7 days of training.

Statistical analysis was conducted using MATLAB (R2018b, Mathworks) or GraphPad Prism 7 (GraphPad). Press rates in VI30, VI60, devaluation probes, LPr, reinstatement day, and change across omission days, as well as distance moved, velocity, and rotations in open field were compared between lesion and control groups with unpaired student’s t-tests. Efficiency was assessed by dividing number of presses or head-entries to number of rewards, which was calculated for day 1 and day 5. Day 5 efficiency was then normalized to day 1 and was compared using a one-sample t-test comparing means to 100% (no change). Finally, press rates across across learning, probe days, omission, performance in rotarod across trials, cross-correlations, and inter-press interval histograms were compared using two-way repeated measures ANOVA. For ANOVAs, the Sidak’s multiple comparisons test was used for post-hoc tests except for histograms and cross-correlation, where a bonferroni corrected multiple comparisons was performed. Significance was defined as p ≤ 0.05.

Results

Lesion of striatal patches enhances behavioral variability
To explore patch contribution to habitual behaviors, we used Sepwl-Cre mice, which express cre-recombinase in patches [34], and an AAV encoding a modified caspase 3 virus to specifically lesion striatal patches. Injection of AAV led to deletion of patches in dorsal striatum (Fig 1A-C). Three weeks after injection of virus (n = 14) or vehicle (n = 15), mice were trained on a variable interval schedule of reinforcement, which has been shown to induce habitual responding in mice ([9], Fig 1D). Both lesioned and control mice increased press rates across FR1, VI30, and VI60 training (two-way repeated-measures ANOVA, significant effect of day, F(8,216) = 24.9, p < 0.0001) and lesioned mice were not impaired in acquisition of the task relative to controls (non-significant effect of group, F(1,27) = 0.2706, p = 0.6071; non-significant interaction, F(8,216) = 1.687, p = 0.9660; Fig 2A). Interestingly, across training, control mice were more consistent in their day-to-day press rates relative to patch lesioned mice. Figure 2B+C show the daily presses of one mouse subtracted from the average press count for that mouse across VI60 training in both a representative control (Fig 2B) and lesioned mouse (Fig 2C). Here, larger bars reflect increased variance across days. Indeed, across VI60 training days, lesioned mice displayed significantly increased behavioral variability in response rates (unpaired t-test, t = 2.797, df = 27, p = 0.0094; Fig 2D). Similarly, press rates in control mice were more predictive of press rates the following day, as they demonstrated significantly greater autocorrelation coefficients (at lag 1) relative to lesioned mice (unpaired t-test: t = 2.144, df = 21, p = 0.0439, Fig 2E). This suggests that lesioning patches may disrupt the stabilization of lever press rate across training, which may indicate increased behavioral flexibility. Despite this, press rates did not differ between patch lesioned or control mice in VI60 trials (t = 0.3034, df = 27, p = 0.7639, Fig 2F). Together, this suggests that lesioning striatal patches does not impair acquisition of VI60 training, though lesions may enhance behavioral variability across days.

Lesioning striatal patches increases response variability. A. Across CRF (FR1), variable interval 30 (VI30), and variable interval 60 (VI60) training, lesion (red) and non-lesion control (blue) mice have similar increases in press rates. B-C. Representative day-to-day variation of press rates for a control (B) and lesioned (C) mouse. The line at 0 represents the mean press count across all VI60 days for each respective mouse and bars represent the difference from the mean on each day. D. Variation in press rates across VI60 training days is significantly increased in lesioned mice relative to controls. E. Autocorrelation coefficient at lag 1 is reduced in patch lesioned mice relative to controls. F. Press rates across all VI60 trials are not different between lesioned and control mice. * indicates p < 0.05.

Lesion of striatal patches alters behavioral strategy and efficiency

Increased behavioral variability suggested that lesioned mice may display other differences in responding across VI
training. Therefore, we plotted distributions of inter-press intervals across both groups in day 1 and day 5 of VI60 training (Fig 3A+B). The distribution of inter-press intervals between groups demonstrated a similar bimodal shape suggesting similar response rates between groups. Over training, control mice tended to increase presses separated by ~2 sec (two-way repeated measures ANOVA, both factors repeated measures, significant interaction, $F_{(500, 5500)} = 1.48$, $p < 0.0001$, significant bonferroni-corrected post-hoc tests shown on figure; Fig 3A), while patch lesioned mice tended to suppress responses at this interval (two-way repeated measures ANOVA, both factors repeated measures, significant interaction, $F_{(500, 4500)} = 1.56$, $p < 0.0001$, significant bonferroni-corrected post-hoc tests shown on figure; Fig 3B). Ultimately, this resulted in a significant increase in efficiency in lesioned mice over training (one-sample t-test, $t = 2.377$, df = 10, $p = 0.0388$, Fig 3C), while control mice displayed no change in press:reward efficiency from day 1 to 5 (one-sample t-test, $t = 0.2779$, df = 11, $p = 0.7862$, Fig 3C). We next repeated this analysis for head entries into the food magazine by plotting inter-head-entry-intervals and comparing efficiency. An even more robust difference emerged by day 5 suggesting that control mice increased head-entries, particularly at the ~2 sec inter-entry-interval (two-way repeated measures ANOVA, both factors repeated measures, significant interaction, $F_{(500, 4500)} = 1.56$, $p < 0.0001$, significant bonferroni-corrected post-hoc tests shown on figure; Fig 3D). On the other hand, lesioned mice tended to reduce head entries separated by ~2 sec, though this effect was not significant (two-way repeated measures ANOVA, both factors repeated measures, non-significant effect of day or interaction). This resulted in a partial increase in head-entry:reward efficiency in lesioned mice (one-sample t-test, $t = 1.917$, df = 10, $p = 0.0842$, Fig 3F) and no change in control mice (one-sample t-test, $t = 0.4354$, df = 11, $p = 0.6717$, Fig 3F). Together, this suggests that control mice develop a less efficient strategy to obtain rewards relative to lesioned mice, potentially due to emergence of habitual, stereotyped pressing and magazine entry at 2 sec intervals across learning.

**Fig 3. Lesioned mice develop a more efficient behavioral strategy.** A-B. Distribution of inter-press interval for lesioned (A) and control mice (B) on VI60 day 1 and day 5. C. Lesioned mice become more efficient (# presses / # rewards) across training, while controls do not. D-E. Distribution of inter-entry-interval for lesioned (D) and control mice (E) on VI60 day 1 and day 5. F. Lesioned mice become slightly more efficient (# head-entries / # rewards) across training, while controls do not. G-H. Cross-correlation of press rate and head entry rate in 100 ms bins for control (G) and lesioned (H) mice (lags -50 to 50; see text for details). # indicates $p < 0.1$; * indicates $p < 0.05$.

Increased efficiency can come from two sources: by increasing effective responding, or from suppression...
of ineffective response patterns. In the context of the variable interval schedule, a single press followed by head-entry is the most efficient strategy to obtain a reward, while head-entries followed by presses are less efficient. Therefore, to characterize response patterns over time, we performed a cross-correlation analysis of presses and head-entries. Briefly, press and head-entry counts were taken across 100 ms bins for day 1 and 5 and presses were correlated to head entry at a range of intervals (lags -50 to 50). Highly correlated responding at lag 0 indicates that presses were predictive of head entries in the same 100 ms bin. Correlation at lag -50 suggests presses were predictive of head entries 5 sec later, and correlation at lag 50 suggests a presses were predictive of head entries occurring 5 sec before pressing. Lags between these extremes represent correlation at a shorter interval between press and entry rates. Between day 1 and 5, control mice show a change in responding with both an increase in correlation between presses-to-head entry, and an increase in headentry-to-press responding (two-way repeated measures ANOVA, both factors repeated measures, significant interaction, $F_{(99,1089)} = 4.232, p < 0.0001$; Fig 3G, significant bonferroni-corrected post-hoc tests shown on figure). This suggests that control mice increase efficient and inefficient sequences of behavior, which is consistent with no change in overall efficiency. On the other hand, lesioned mice subtly modify their responding across training, with an increased correlation in short press-to-headentry responding (two-way repeated measures ANOVA, both factors repeated measures, significant interaction, $F_{(99,990)} = 3.545, p < 0.0001$; Fig 3H, significant bonferroni-corrected post-hoc tests shown on figure). Thus, while control mice increase both efficient press-check responses and inefficient response patterns that may be reflective of reflexive, stereotyped head-entries, lesioned mice never develop this latter behavior and improve their press-to-check responding, which improves overall efficiency. This improvement may suggest that patch lesioned mice maintain goal-directed responding across learning, while controls develop habitual, inefficient response patterns.

**Lesion of striatal patches disrupts habitual behaviors**

Habitual behavior is operationally defined by resistance to outcome devaluation; that is, habitual organisms will continue to respond for a reinforcer even after being given free access to the reinforcer [38]. Thus, after the completion of training, mice were given free access to either home chow (valuation condition) or the sucrose reward they received in the operant task (devaluation condition), randomized across two days (Fig 1D). We did not note a significant effect of patch lesions across devaluation trials (unpaired t-test, $t = 1.298, df = 27, p = 0.2054$; Fig 4A). We next quantified habitual behavior by normalizing lever press rate in devaluation trials to press rates in valuation
trials (LPr, see [37]) to compare the effects of reward-specific valuation to generalized satiation. Similar to
devaluation trials, this metric was also not different between lesioned and control mice (unpaired t-test, t = 0.09028,
df = 21, p = 0.9289; Fig 4B). However, we did observe a significant decrease in lever pressing across probe days
(two-way repeated-measures ANOVA, significant effect of day, F(1,21) = 21.38, p < 0.0001; Fig 4C), demonstrating
that mice tended to decrease pressing across days similarly between lesion and control mice (non-significant effect
of group, F(1,21) = 0.0156, p = 0.9018, no significant interaction F(1,21) = 0.1939, p = 0.6642). This finding is not
consistent with prior reports [37] and indicate that Sepw1 mice rapidly extinguish responding across subsequent
probe trials. Due to the effect of day occluding any effect of probe condition, we were unable to draw conclusive
inferences about the degree of habit formation from these data.

300

Fig 4. Lesions of striatal patches do not change devaluation, but do reduce omission responses. A.
Devaluation press rates normalized to all VI60 training days is not different between lesion (red) and control mice
(blue). B. Devaluation press rates normalized to valuation press rates (LPr, see text) did not differ between lesioned
and control mice. C. Lesioned and control mice both decrease response rates across subsequent probe days. D.
Lesioned mice increased responding to a greater extent than controls during reinstatement to the VI60 schedule. E.
Mice then underwent omission across two days. Lesioned mice had reduced press rates relative to controls in day 1
of omission. F. Press rates within the first and second half of omission day 1 suggest reduced responding in lesion
mice during the first half relative to control mice. * indicates p < 0.05.

309

Since this effect of time complicates interpretation of devaluation results, we next retrained mice with one
additional day of VI60 (reinstatement) to reestablish high press rates. We then performed two days of omission as a
further assessment of habitual responding. Here, the press contingency was reversed and mice were rewarded every
20 seconds if they refrained from lever pressing, and any presses reset this timer. This approach is more efficient at
extinguishing behaviors than extinction, and can be used to assess strong habits [19]. Lesioned mice reinstated lever
pressing to a greater extent than control mice during reinstatement (unpaired t-test, t = 2.698, df = 27, p = 0.0119;
Fig 4D), further indicating enhanced behavioral flexibility. During omission, lesioned mice demonstrated
diminished press rates relative to control mice (two-way repeated-measures ANOVA, significant time x group
interaction, F(1,27) = 5.17, p = 0.0311; Fig 4E), suggesting habitual responding is impaired in these mice. Post-hoc
tests revealed that control mice had elevated press rates on the first day of omission compared to lesioned mice
(Sidak’s multiple comparisons test, Day 1, p = 0.0288). We next analyzed the press rates within the first and second
halves of this first omission trial. Both lesioned and control mice tended to decrease their press rate over time (two-
way repeated-measures ANOVA, significant effect of time, F(1,27) = 83.76, p < 0.0001) though lesioned mice had
suppressed response rates over both halves (significant effect of group, $F_{(1,27)} = 6.028$, $p = 0.0208$, no group x time interaction, $F_{(1,27)} = 0.7304$, $p = 0.4003$). A subsequent post hoc Sidak’s multiple comparison test revealed a significant difference between lesioned and control mice during the first half of omission ($p = 0.028$). Reduced press rates in lesioned mice during omission suggests that they form weaker habits, indicating that lesioning patches interferes with normal habit formation.

Lesion of striatal patches impairs motor learning, but not locomotion

Deficits in operant conditioning may be due to differences in habit formation or to generalized motor deficits. Therefore, after the completion of variable interval training, we assessed the effect of lesioning patches on motor learning using an accelerating rotarod. Mice performed four trials per day for four days, and latency to fall was measured (maximum 360 seconds; [36]). Both lesioned and control mice increased performance across days, as indicated by a significant effect of day (two-way ANOVA with multiple comparisons, main effect of day: $F_{(3,81)} = 49.58$, $p < 0.0001$). However, no effect of lesion was noted across all four tested days (non-significant effect of group: $F_{(1,27)} = 2.119$, $p = 0.1570$, non-significant interaction, $F_{(3,81)} = 1.513$, $p = 0.2173$). A Sidak’s multiple comparisons test indicated that performance on day 1 was significantly different between lesioned and control mice ($p = 0.0452$; Fig 5A). Within the first day of testing, lesioned and control mice improved performance (two-way repeated-measures ANOVA, significant effect of trial, $F_{(3,81)} = 12.54$, $p < 0.0001$) though lesioned mice were slightly impaired relative to controls as indicated by a trending effect of group ($F_{(1,27)} = 3.944$, $p = 0.0573$; Fig 5B). Post-hoc Sidak’s multiple comparisons test revealed significant differences between groups on trials 2 ($p = 0.0427$) and 3 ($p = 0.0456$). However, by day 4, this difference was not present (two-way repeated-measures ANOVA, non-significant effect of group, $F_{(1,27)} = 0.1248$, $p = 0.7267$, Fig 5C) and performance stabilized (non-significant effect of time, $F_{(3,81)} = 0.2656$, $p = 0.7627$). This indicates that lesion of patches may disrupt initial motor learning, but with time, patch-lesioned mice were able to perform at the same level as control mice.

Fig 5. Patch lesions impair motor learning but not overall performance or locomotion. Performance on the rotarod across days. Lesioned mice are impaired on day 1 of rotarod learning. B. Performance on each trial within day 1 of rotarod training suggests that lesioned mice are impaired in the second and third trials relative to control mice. C. Performance on each trial within day 4 of rotarod training suggests that lesioned mice perform similarly late in training. D-F. Performance in open field suggests that lesioned mice are not different than controls in the overall distance moved (D), overall velocity (E), or in number of rotations (F). * indicates $p < 0.05$.  

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To assess overall motor activity, a subset of mice \( n = 13 \) control, \( n = 11 \) lesion) were placed in an open field and distance moved, velocity, and rotations were quantified. We observed no differences in overall movement (unpaired t-test, \( t = 0.7784, df = 22, p = 0.4446 \)), average velocity (unpaired t-test, \( t = 0.7835, df = 22, p = 0.4417 \)), rotation (unpaired t-test, \( t = 0.1968, df = 22, p = 0.8458 \); Fig 5D-F). These data indicate that patches may play a role in early motor learning, but that lesioning patches does not affect motor functioning.

Discussion

Here, we investigated a role for striatal patches in habit formation and motor behaviors. To do this, we selectively lesioned patches using a Cre-dependent caspase 3 virus in Sepw1 NP67 mice, we noted loss of striatal patches. Mice with patch lesions demonstrated normal learning on a variable interval task, but displayed greater day-to-day variability in response rates across training. Further, control mice developed aspects of habitual responding during training, while lesioned mice did not, resulting in increased efficiency in lesioned mice. Lesioned mice did not display impaired devaluation press rates, though this result is complicated by a generalized decrease in response rates across valuation and devaluation probe days. Lesioned mice also suppressed press rates faster than control mice when they were placed on an omission task, where responses had to be withheld to earn rewards. Taken together, these results indicate that patch lesioned mice demonstrated weakened habitual behaviors and impaired behavioral stability across training and changes in task design, suggesting that striatal patches may be a key site of behavioral stability. Finally, patch lesioned mice showed slight impairment in acquisition of a new motor skill on a rotarod and no impairments in baseline locomotor activity, suggesting patches may regulate motor learning, but not motor execution per se, and that deficits in operant behaviors are not simply attributable to motor deficits.

In the current study, we noted that patch lesions impaired habitual responding during omission trials, where mice had to suppress response rates to obtain rewards (Fig 4E+F). Omission is a robust means of extinguishing habitual behaviors [19], and the tendency of patch-lesioned mice to rapidly suppress response rates suggests impaired habit formation. This is consistent with a recent study that used a conjugated cytotoxin (dermaphorin-saporin) to selectively ablate \( \mu \)-opioid neurons in the striatum and that found that habit formation was impaired [33]. These findings are also consistent with studies suggesting lesions of patches impair inflexible motor stereotypies [31,32]. Jenrette et al. noted deficits in press rates when sucrose rewards were paired with lithium chloride to devalue sucrose rewards through taste aversion. However, the current study did not find a deficit in devaluation
press rates when mice were provided free access to sucrose. We attribute this difference to two main factors. First, the method of devaluation (free access to reward vs. taste aversion) may not similarly devalue rewards, and it is possible that taste aversion is a more robust manipulation. Second, we noted a significant effect of probe day such that mice pressed less on day 2 regardless of probe condition (Fig 4C), indicating that the counterbalancing of days confounded any effects of probe condition. The reasons for this remain unclear, as multiple papers have successfully used this probe paradigm to assess habitual behavior [20,37]. One potential explanation is that Sepw1-Cre mice may have differences in instrumental learning relative to mice used in the aforementioned experiments. Indeed, expression of Cre-recombinase has the potential to alter behaviors [39].

It remains unclear how patches encode habitual behaviors. It is likely that disruption of striatal patches leads to over-reliance on brain circuits subserving goal-directed behaviors, including the prefrontal cortex, nucleus accumbens and dorsomedial striatum [33]. Activity in striatal patches is tied to reward processing [40,41], and patches support intracranial self-stimulation [42], suggesting that patches have a role in reinforcement. Patch spiny projection neurons also have direct inputs to dopamine neurons [25–27] and a recent dissertation indicates they may suppress dopamine activity through GABA_A-mediated inward currents [43]. Lesions to patches may therefore influence spiraling basal ganglia circuits [44] by causing dysregulation of striatal dopamine release that may manifest as impaired reinforcement or disrupted decision making processes [45,46]. Indeed, dopamine signaling shifts from ventromedial to lateral striatum with extended training [47], and this process may be impacted by lesions to patches. Future studies should examine the interplay between patches and dopamine across habit formation to explore this possibility.

Alternatively, patches may mediate habitual behaviors through the endocannabinoid system in the striatum. CB1 receptors are crucial for striatal plasticity and synaptic depression [48,49], and these receptors are enriched in both striatal patches [50] and in striatal projections from the orbitofrontal cortex [13]. Indeed, the orbitofrontal cortex is thought to be key in habit and cognitive flexibility [51,52], and orbitostrial projections are key in habit formation and transitions from goal-directed to habitual strategies [12,53]. Further, knockout of CB1 receptors from orbitostrial terminals impairs habit formation [13]. Thus, CB1 receptors are in a prime position to mediate habit-related plasticity in striatal patches. Loss of striatal patches might impair this process, which may disrupt the transfer from goal-oriented to habitual behavior.

An unexpected finding from the current work was increased day-to-day behavioral variability in patch lesioned
mice (Fig 2B-E). These data suggest that lesions of striatal patches may generally increase behavioral variability across days. This could be due to two factors. First, this could reflect an impairment in reward-related memory reconsolidation, which would suggest patches may store reward-related information. Alternatively, patches may generally play a role in regulating crystallization of motor patterns, thus establishing habits. Many organisms crystalize motor patterns beyond habit formation in operant conditioning: across development, seasons, or lifespan. For example, many species of songbird show elevated variability in song production either as juveniles or during winter seasons; this variability is eventually reduced over time [54]. Indeed, the basal ganglia is thought to modulate variability in song production in birds [55]. Moreover, spiny projection neuron distribution and patch organization differ between vocal and non-vocal songbird species [56]. Similarly, in rodents, spontaneous variation in foraging patterns are common, even following reinforcement of prior exploration (a win-shift pattern, [57,58]). Non-specific lesions of dorsal striatum impair this behavioral variability and can increase spontaneous alternation in ‘win-stay’ conditions, where rodents need to return to previously rewarded areas [59,60]. Future studies could investigate striatal patches as a site for stabilizing behavioral patterns in motor behaviors and reinforcement learning beyond operant conditioning.

While habitual strategies free cognitive resources are therefore more efficient overall, goal-directed animals are sensitive to reward outcomes and might be more likely to optimize their behavioral strategy. Indeed, here, control mice begin making more stereotyped presses and head-entries and increase head-entry-to-press sequences over training, establishing an inefficient, habitual checking strategy (Fig 3). On the other hand, mice with lesioned patches fail to establish this checking behavior and suppress unnecessary presses and head-entries, resulting in an increase in efficiency. Repetitive head-entries may result in overtraining, which could enhance the establishment of inflexible responding [61]. On the other hand, the propensity of control mice to develop these behaviors may be reflective of ongoing habit formation, that is, repeated head-entries may be a marker of the establishment of habits, which is disrupted in mice with lesioned patches. Indeed, several differing views have emerged regarding why habits develop. First, it is thought that repeated pairings of behavior an reward result in habits [62]. Alternatively, tasks where the link between action and outcome is more difficult to predict drives habitual responding, explaining why random ratio schedules maintain more goal-directed responding relative to random interval schedules [61]. A related, but novel idea has been recently put forward: that tasks where animals are able to pay less attention to their responding and the outcome of behavior may drive habits [63]. Here, sham controls may be able to pay less attention
to their responding due to the autonomy afforded by intact patches, while lesioned mice must attend to outcomes, resulting in efficient and goal-directed behavior. Future studies utilizing variable interval schedules of reinforcement should investigate changes in responding during training that might predict habit formation.

Consistent with previous reports [64], patch lesioned mice also have deficits in early motor learning, but not in general movement parameters (Fig 5). Notably, minor dopamine dysfunction also leads to deficits in motor learning, but not general motor deficits [65], again raising the possibility that these deficits are partially mediated by dysfunctional dopamine regulation following patch lesions. Indeed, recent work suggests that patch lesions may drive dopamine dysfunction in the striatum, which may directly affect early motor learning [66]. Despite deficits in learning on the rotarod, it remains unlikely that motor learning is the only function of patch compartments, as our results also suggest learning of lever-pressing, locomotion, and final performance on rotarod all remain intact following patch lesion. Other studies investigating fine motor control have found that selective inhibition of matrix neurons using DREADDs disrupts performance in reaching and grasping tasks [67]. Patch compartments have been better studied in decision making [45,46] and reward processing [40,41]. Together, this suggests that matrix neurons may regulate motor execution, whereas patch neurons regulate timing and selection of actions. Indeed, this notion is consistent with computational models [68], which hold that patches bias matrix neurons towards specific actions.

In sum, this work adds to a growing literature suggesting striatal patches support habit formation [29,33]. Lesioning patches may lead to overactivation of brain structures that support goal-oriented behaviors, including the dorsomedial striatum or prefrontal cortex [19,51]. Alternatively, patch lesions may alter dopamine signaling in striatum [25,27]. Finally, brain regions supporting inflexible behaviors have been implicated in the pathology of Obsessive Compulsive Disorder [2–4], drug addiction [5–7], and Tourette’s Syndrome [8]. Future studies should investigate the contribution of striatal patches to these disease states.

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**Author Contributions**


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Figure 4

(A) Devaluated Press Rate (%)

(B) Norm Devalued LPr

(C) Devaluated Norm Press Rate (%)

(D) Reinstatement Press Rate (%)

(E) Omission Press Rate (%)

(F) Omission Press Rate (%)

Probe Day

Omission Day

Half of Omission Day

Day 1

Day 2
Figure 5
Figure 2
Figure 1
Figure 3