Acquisition of Auditory Discrimination Mediated by Different Processes through Two Distinct Circuits Linked to the Lateral Striatum

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Abstract

The striatum, the central hub of cortico-basal ganglia loops, contains functionally heterogeneous subregions distinguished by the topographic patterns of structural connectivity. These subregions mediate various processes of procedural learning. However, it remains unclear when and how striatal subregions engage in a new learning of sensory stimulus-based decision-making. Here, we show that the anterior dorsolateral striatum (aDLS) and posterior ventrolateral striatum (pVLS) are activated in a different temporal pattern during the acquisition phase of auditory discrimination learning. The aDLS promotes the behavioral strategy by the stimulus-response association while suppressing those driven by the response-outcome association, and the pVLS is involved in forming and maintaining the stimulus-response strategy. These two subregions exhibit distinct event-related neuronal responses during the learning processes. Our findings demonstrate that aDLS and pVLS neurons integrate the new learning of auditory discrimination in spatiotemporally and functionally different manners.

Introduction

The neural network connecting the cerebral cortex, basal ganglia, and thalamus forms functionally heterogeneous loops distinguished by the topographic patterns of connectivity[1,2], and is implicated in various learning processes by the integration of cognitive, sensorimotor, and reward information[3,4]. The striatum is a key node in the cortico-basal ganglia loops, and its segregated subregions are considered to mediate procedural learning, such as motor skills and instrumental behavior, through parallel processing via different loops[5-7]. Previous studies in humans and non-human primates have reported that the neural activity within the striatum shifts from the associative subregions (caudate and anterior putamen) to the sensorimotor subregions (posterior putamen) as motor learning progresses[8-11]. In rodents, an instrumental behavior initiates as goal-directed actions and then transits to stimulus-response habits[12,13], requiring the dorsomedial striatum (DMS; a homologue to the caudate in primates) and the dorsolateral striatum (DLS; a homologue to the putamen in primates), respectively[14,15]. Based on these results, a model of procedural learning has been proposed, in which the functional dominance changes from the associative to sensorimotor subregions in the striatum during the learning phases[16].

In contrast, deviations from the prior model are evident in other behavioral tasks on decision-making based on external sensory cues. For example, the neural activity in the caudate in humans is associated with learning of a visuomotor association task and the execution of learned behavior[17,18]. The tail of the coudate in non-human primates contributes to the automatic, habitual behavior in decision-making task using complex visual stimuli[19]. In rodents, the DLS is involved in the acquisiton and execution of the discrimination task with visual and auditory cues, wheras the DMS madiates the performance of learned behavior[20,21]. Moreover, other striatal subregions, such as the posterior striatum and ventrolateral striatum in rodents are also engaged in the auditory discrimination[22-24]. Based on these results, a hypothesis has arisen that the acquisition of sensory cue-based decision-making requires wide-range spatiotemporal processing within the striatum. However, the neural mechanism by which the functional circuits linked to striatal subregions change throughout learning processes is not yet fully understood.

To investigate a large-scale striatal reorganization in accordance with the acquisition of external cue-dependent decisions, we conducted a two-alternative auditory discrimination task[25]. Using a small-animal neuroimaging technique, we found that the anterior dorsolateral striatum (aDLS) and posterior ventrolateral striatum (pVLS) are activated to the highest level at the middle and late stages during the acquisition phase of auditory discrimination, respectively. Then, chronic pharmacologic manipulations confirmed that the aDLS and pVLS are necessary for discrimination learning, and the transient manipulations also ascertained the function of

these subregions in the learning at the corresponding stages. The resultant effects of the manipulations on the behavioral strategy showed that the aDLS promotes the strategy based on the stimulus-response association, while suppressing those based on response-outcome associations. Additionally, the pVLS engages in the formation and maintenance of the stimulus-response association strategy. Electrophysiological recording of striatal neurons indicated that subpopulations of aDLS neurons mainly represent the outcome of specific behaviors at the initial period of discrimination learning, whereas pVLS subpopulations encode the beginning and ending of each behavior in association with the progress of the learning. In addition, other subpopulations of aDLS and pVLS neurons show sustained activation after obtaining reward with distinct patterns of the combination between the stimulus and response. These findings reveal that the aDLS and pVLS regulate the acquisition of auditory discrimination through distinct spatiotemporal and functional manners, challenging the prior model which proposed a transition from the associative to sensorimotor striatal subregions during learning.

Results

Leaning processes of auditory discrimination

We employed a two-alternative auditory discrimination task, in which animals are required to make new associations between a tone instruction cue (2 or 10 kHz) and a response (pressing the left or right lever)[25], setting the interval between the stimulus presentation and lever insertion to 3 s (Fig. 1a). The success rate gradually increased, reaching a plateau around Day 13, which persisted through Day 24 (Fig. 1b). When the success rates across Days 2, 6, 10, 13 and 24 were compared, significant differences were observed between Days 2 and 6, Days 6 and 10, as well as Days 10 and 13, showing no significant difference between Days 13 and 24 (Fig. 1c). In contrast, the response time and omission rate did not vary among the training days (Fig. 1d,e), and the variation of response bias decreased along with the progress of auditory discrimination (Fig. 1f). Based on changes observed in the success rate, we categorized the learning processes into two phases: the acquisition phase, which lasted until Day 13; and the learned phase, which began after Day 13.

Distinct brain activity patterns in striatal subregions during learning processes

To investigate dynamic changes in regional brain activity in the entire striatum during auditory discrimination in the same animals, we conducted the positron emission tomography (PET) for small animals with 2-deoxy-2-[^{18}F]fluoro-$_{D}$-glucose (^{18}F-FDG), which measures cerebral glucose metabolism correlated to brain activity[26-28]

(Fig. 2a). We performed ^{18}F-FDG-PET scanning on Day 4 of the single lever press task without sound-based decision as a control condition and during a series of the trainings including the acquisition (Days 2, 6, and 10) and learned (Day 24) phases (Fig. 2b). The average number of lever presses in the single lever press task exceeded 80 times (81.6 ± 1.3, mean ± s.e.m.) on Day 2 and maintained that level until Day 4 (Fig. 2c). In the following auditory discrimination task, the success rate gradually increased along with the training days (Fig. 2d), showing a significant difference among sessions in which we performed the ^{18}F-FDG-PET scanning (Fig. 2e). The response time and omission rate were consistent among the days, and the variation of response bias became smaller as the task progresses (Fig. 2e). These performances were similar to those in the intact rats (compared to Fig. 1c–f), suggesting that the procedure for ^{18}F-FDG-PET scans does not affect the acquisition of discrimination.

The regional brain activity related to the behavioral task was analyzed with a voxel-based statistical parametric analysis[26], in which the ^{18}F-FDG uptake on either day of the discrimination task was compared with that on the single lever task. The task-related brain activity in the whole brain is summarized in Extended Data Table 1. In the present study, we focused on the task-related activity in striatal subregions. We found significant increases of the activity in the unilateral aDLS and the bilateral pVLS on Days 6 and 10 during the acquisition phase (Fig. 2f,g). However, no significant change was observed in the DMS during the acquisition phase except a unilateral decrease on Day 24 (Fig. 2f). Next, to evaluate the activity related to the progress of learning, ^{18}F-FDG uptake on Days 6, 10, and 24 was compared with that on Day 2. The learning-dependent brain activity was significantly increased in the bilateral aDLS and the unilateral pVLS on Days 6 and 10, respectively (Fig. 2h), whereas the activity decreased in the bilateral DMS on Day 24 (Fig. 2h). This decrease was also detected when ^{18}F-FDG uptakes were compared between Days 10 and 24 (Fig. 2h), suggesting the down-regulation of the DMS activity in the learned phase.

To quantitatively validate changes in the brain activity in striatal subregions, we analyzed the amount of ^{18}F-FDG uptake in the voxel of interest of these subregions (Fig. 2i). The ^{18}F-FDG uptake in the bilateral aDLS reached a peak on Day 6, and it decreased during the subsequent days (Fig. 2j), whereas the uptake in the bilateral pVLS gradually increased along with the progress of learning, showing the maximal value on Day 10 (Fig. 2k). The uptake in the DMS did not alter during the acquisition phase, and it decreased on Day 24 (Fig. 2l). These results suggest that the aDLS and pVLS contribute to learning processes in a different temporal pattern during the acquisition phase, whereas the DMS may be engaged to the execution of the behavior in the learned phase.

Excitotoxic lesion of the aDLS and pVLS, but not the DMS, disrupts the acquisition of auditory discrimination

To study whether the changed activity of striatal subregions is involved in learning processes, rats received a bilateral injection of solution containing ibotenic acid (IBO; 8 mg/mL, 0.3 μL/site) or phosphate buffered saline (PBS) into the subregions, and we conducted for the behavioral study. The range of IBO lesion in the subregions was analyzed by immunostaining with a neuronal marker NeuN after the behavioral tests (Fig. 3a–c). The number of single lever presses increased similarly along with the training in both the PBS- and IBO-injected groups into the aDLS, pVLS, or DMS along with the training (Fig. 3d–f). In the discrimination learning, increases of the success rate were impaired in both IBO-injected aDLS and pVLS groups, compared to the corresponding PBS-injected groups (Fig. 3g,h). The response time in the aDLS-lesioned group did not alter during the discrimination training, whereas that in the pVLS-lesioned group was continuously prolonged (Extended Data Fig. 1a,b), and the omission rate and response bias were similar between the lesioned and the corresponding control groups (Extended Data Fig. 1d,e and 1g,h). In contrast, the success rate in the discrimination task was comparable between the PBS- and IBO-injected groups into the DMS (Fig. 3i). The DMS lesion lengthened the response time through the learning (Extended Data Fig. 1c), whereas it did not alter the omission rate or response bias (Extended Data Fig. 1f,i). These results indicate that the aDLS and pVLS are required for the acquisition of auditory discrimination, whereas the DMS contributes to the execution but not learning of the discriminative behavior, being consistent with previous reports[29,30].

Previous studies have reported that the dorsal striatum is involved in both behavioral strategies based on the response-outcome association and the stimulus-response association during stimulus-response learning[12,13]. To assess the impact of striatal lesions on the behavioral strategies, we analyzed the proportion of response attributed to two representative strategies in all responses of each session. One is the “win-shift-win (WSW)” strategy, in which, after a correct response in the previous trial, rats press the opposite lever in the current trial in response to a shift of the instruction cue, resulting in the correct response. The WSW is considered to reflect the behavioral strategy based on the stimulus-response association[31,32]. Another strategy is the “lose-shift-lose (LSL)” strategy, in which, after an error response in the previous trial rats press the opposite lever in the current trial despite a shift of the instruction cue, leading to another error response. The LSL is considered to appear as a consequence of the behavioral strategy based on the response-outcome association[31]. In the PBS-injected group into either of three striatal subregions, the proportion of WSW and LSL strategies increased and decreased, respectively, during the acquisition phase (Fig. 3j–l). In the IBO-lesioned group into the aDLS, the increase in the WSW proportion was

markedly impaired as compared to the corresponding control group, and the decrease in the LSL proportion was delayed compared to the same group (Fig. 3j). In the pVLS-lesioned group, the increasing WSW proportion was also impaired as compared to the corresponding controls, although the change in the LSL proportion was comparable to the control response (Fig. 3k). In contrast, in the DMS lesion group both WSW and LSL proportions did not show significant differences from the corresponding values in their control group (Fig. 3l). These results indicate that the aDLS promotes the WSW strategy through stimulus-response association and suppresses the LSL strategy rooted in response-outcome association, and that the pVLS selectively facilitates the WSW strategy acquisition. Thus, these two subregions not only play roles in the acquisition of auditory discrimination and but also control distinct behavioral strategies during the learning processes.

After the completion of discrimination training, we tested locomotion and feeding behavior of rats with aDLS and pVLS lesions. There was no difference in locomotor activity in the open field and the amount of food intake in both lesioned groups (Extended Data Fig. 1j–m), suggesting that learning deficits following excitotoxic lesions of the striatal subregions cannot be attributable to abnormalities in these general motor behaviors.

Inhibiting aDLS and pVLS activity impairs discriminative behavior at different stages of the acquisition phase

Our ^{18}F-FDG-PET imaging study indicated that the brain activity in the aDLS and pVLS was increased in a different temporal pattern during the acquisition phase of auditory discrimination. To examine whether neuronal activity in striatal subregions is linked with the processes at different stages, we temporarily inhibited the activity in each subregion by the treatment with a $GABA_A$ receptor agonist muscimol (MUS). In a pilot study, we tested the effect of the bilateral injection of several doses of MUS (0.1, 0.2, and 0.5 μg/μL) into the aDLS or pVLS on the single lever press behavior and confirmed that the administration of the lowest dose of MUS did not affect the frequency of single lever presses and the quantity of food intake (Extended Data Fig. 2a–d). In addition, we divided the acquisition phase into three stages based on the success rate that included the early (< 60%), middle (60–80%), and late (80–100%) stages. To achieve the stage-specific inactivation of the aDLS or pVLS neurons, rats were given bilateral injections of MUS or saline (SAL) at three different timings during a series of the training. The first injection was conducted on Day 2 to target the early stage. The second and third injections were conducted on the day after the success rate had reached 60% and 80% for the first time through the training, respectively, to target the middle or late stage. After the injections, the rats were used for the behavioral test. Placement locations of the injection cannula into the striatal subregions were assessed by cresyl violet staining

after the behavioral tests (Fig. 4a,b). The success rates on the day before the injection (day N-1) at the early, middle, and late stages were similar between the two groups that received the bilateral injections of MUS or SAL (Extended Data Fig. 2e,f). Rats were then administered injections into the aDLS on the next day (Day N), and the success rate in the MUS-injected groups significantly decreased at the middle stage compared to the corresponding SAL-injected groups, but not at the early and late stages (Fig. 4c, left). In contrast, the success rate in the MUS-injected groups into the pVLS significantly decreased at the late stage, but not at the early and middle stages, compared with the respective SAL-injected groups (Fig. 4d, left). To normalize the variation of learning speed in the individual animals, we calculated the value by subtracting the success rate on Day N-1 from that on Day N in the individual animals. There were significant reductions in the subtracted rate at the middle stage in the MUS-injected group into the aDLS (Fig. 4c, right) and at the late stage in the MUS-injected group into the pVLS (Fig. 4d, right). These results indicate that the aDLS and pVLS mainly function at the middle and late stages, respectively, in the acquisition phase of auditory discrimination, supporting the results obtained from our imaging study.

Next, we analyzed the influence of temporal striatal inactivation on the behavioral strategy at the middle and late stages of the acquisition phase. We calculated the proportion of WSW and LSL strategies, and subtracted the values of WSW or LSL proportion on Day N-1 from those on Day N. The proportion of both strategies on Day N-1 were comparable between the MUS- and SAL-injected groups (Extended Data Fig. 2g,h). In the aDLS, the subtracted proportion of LSL strategy at the middle stage significantly increased in the MUS-injected group as compared to the SAL-injected group, although the subtracted proportion of WSW strategy tended to decrease in the MUS-injected group as compared to the control group (Fig. 4e), and the subtracted proportion of WSW or LSL strategy at the late stage was similar between both groups (Fig. 4f). In the pVLS, the subtracted proportions of the WSW and LSL strategies at the middle stage were similar between the MUS- and SAL-injected groups (Fig. 4g), and the subtracted WSW proportion at the late stage significantly decreased in the MUS-injected group as compared to the SAL-injected group, although the subtracted LSL proportion was comparable between the two groups (Fig. 4h). Our results show that transient inhibition of aDLS activity at the middle stage affects the LSL strategy and has a moderate effect on the WSW strategy, supporting that the aDLS mediates the acquisition process of auditory discrimination at the middle stage at least partly through the suppression of the response-outcome association. Although the impact of inhibition on the WSW strategy was modest, excitotoxic lesion of the aDLS influenced the WSW strategy in addition to the LSL strategy (see Fig. 3j), suggesting that the aDLS acts to enhance the auditory discrimination by promoting the stimulus-response association, together with the

suppression of the response-outcome association. The discrepancy in the extent of changes in the WSW strategy between the two treatments may be generated from the difference in the timing or sustainability of drug inhibition of the striatal subregion. In addition, our results show that pVLS inactivation at the late stage selectively impairs the WSW strategy, supporting contribution of the pVLS to the process via progression of the stimulus-response association.

Neuronal activity in the aDLS and pVLS during the acquisition phase

To examine the firing activity in striatal subregions during the acquisition phase of auditory discrimination, we simultaneously recorded neuronal activity in the aDLS and pVLS at the unilateral (left) side by using a multi-unit recording procedure (Fig. 5a). Using immunostaining for tyrosine hydroxylase (TH) after the recordings, we verified the location of electrodes within the striatal subregions (Fig. 5b). After the single lever press task, we conducted the auditory discrimination task for the recordings. In correct trials, the delay period (0.5 ± 0.2 s) was inserted between a lever press and a sound indicating the reward to distinguish neuronal activity related to the two events (Fig. 5c). The behavioral data in individual rats were divided into three grouped sessions corresponding to the early, middle, and late stages, based on a sigmoid function analysis[33] of the success rate in the task. The success rates in the grouped sessions were 52.7 ± 0.8 %, 61.6 ± 0.7 %, and 85.3 ± 1.7 % (mean ± s.e.m.) for the early, middle, and late stages, respectively, and the rate was significantly increased throughout the three stages (Extended Data Fig. 3a). However, the response time, omission rate, and response bias were similar among the stages (Extended Data Fig. 3b–d). Thus, these stages corresponded to the timing window defined in the acquisition phase of the transient MUS inhibition experiment of neuronal activity.

We identified 1,062 and 423 well-isolated neurons from the aDLS and pVLS, respectively, across the three stages (Extended Data Fig. 3e) and focused on the major population of striatal neurons, putative medium spiny neurons (Extended Data Fig. 3f,g). The total numbers of MSNs at the early, middle, and late stages were 295, 364, and 360 neurons for the aDLS, and 87, 110, and 190 neurons for pVLS, respectively. Based on the combinatorial pattern of the tone instruction cue and lever press in our discrimination task, we categorized the electrophysiological data into four trial types: (1) high-frequency tone/right lever press (HR) and (2) low-frequency tone/left lever press (LL) as correct responses; and (3) low-frequency tone/right lever press (LR) and (4) high-frequency tone/left lever press (HL) as error responses (see Fig. 1a). We identified HR or LL type neurons showing significant changes in firing rate related to the instruction cue onset (CO), choice response (CR), reward sound (RS), or first licking (FL) as compared to the baseline firing rate in each of two trials (Mann-

Whitney U test, $p < 0.01$). These neurons were further divided into two groups based on increased or decreased activity (Extended Data Table 2 and Extended Data Fig. 4a,b). In the following analyses, we focused on the HR and LL type neurons with increased event-related activity to explore the firing patterns of neurons underlying behavioral processes of auditory discrimination.

Subpopulations of aDLS neurons, but not of pVLS neurons, show firing activity related to the behavioral outcome

Previous studies have reported that neurons in the dorsal striatum show the outcome-related activity in the instrumental conditioning tasks[24,34]. We thus investigated whether aDLS and pVLS neurons exhibit a response related to the behavioral outcome during the acquisition of auditory discrimination. In the aDLS, the firing activity of RS-related HR type (RS-HR) neurons showed a higher extent in the increase during a period, when the RS is presented in the correct responses, in the HR trial as compared with the LR, LL, or HL trial throughout the three stages (Fig. 5d, top rows). The rate of these neurons also moderately increased during the same period in the LR trial compared to the LL or HL trial across the three stages, although the extent of the increase was lower than that in the HR trial (Fig. 5d, top rows). The area under the receiver operating characteristic (auROC) values revealed a significant difference of the HR trial from the LR trial during the RS period, whereas the values of HL and LL trials indicated a significant difference in the opposite direction from the LR trial during the same period (Fig. 5d, bottom rows). The averaged firing rate during the RS period in the HR or LR trial appeared to modestly increase at the middle stage, but no significant difference existed among the stages (Fig. 5e). To directly compare the number of neurons showing event-related activity among different stages, we calculated the number of RS-HR neurons by matching the trial number at each stage (Fig. 5f). The proportion decreased progressively from the early to middle stages, and to the late stage. In addition, the firing rate of RS-related LL type (RS-LL) neurons exhibited a higher increase in the LL trial and a modest increase in the HL trial while the averaged rate during the RS period was similar among the three stages. Furthermore, the proportion of RS-LL neurons reduced progressively through the stages (Extended Data Fig. 5a–c). These results indicate that the aDLS contained neurons related to the outcome of choice, showing a greater increase in the firing activity for the correct responses (HR and LL trials) than the error responses (LR and HL trials), and that the proportion of these subpopulations was gradually reduced through the progress of learning.

In contrast, during the RS period at all stages, the firing rate and auROC values of RS-HR and RS-LL neurons in the pVLS revealed no significant differences across the four kinds of trial (Extended Data Fig. 5d,e),

285 indicating that the firing activity of pVLS neurons did not appear to be associated with the behavioral outcome.

286

287 **Subpopulations of aDLS and pVLS neurons show sustained firing activity after the reward in different**

288 **manners**

289 The dorsal striatum is known to demonstrate a response of neuronal activity after the positive outcome in the

290 instrumental behaviors[35,36]. We thus examined whether aDLS and pVLS neurons show the response after

291 obtaining the reward during the acquisition phase of auditory discrimination. In the aDLS, the firing rate of FL-

292 related HR type (FL-HR) neurons indicated a sustained elevation after the FL in the HR trial as compared with

293 any of the other three trials across the early, middle, and late stages (Fig. 6a, top rows). The auROC values

294 revealed a significant difference of the LR, LL, or HL trial from the HR trial across the three stages (Fig. 6a,

295 middle rows). In addition, we calculated the averaged firing rate and increased firing period, defined as the total

296 number of time bins above 3 s.d. of the baseline firing rate for 5 s after the FL in the HR trial. The averaged firing

297 rate was comparable between the early and middle stages, but the rate was significantly lower at the late stage

298 compared to either of the other two stages (Fig. 6b). The increased firing period was also comparable between the

299 early and middle stages, showing a significant reduction at the late stage (Fig. 6c). The proportions of FL-HR

300 neurons were similar between the early and late stages, although they showed a slight decrease at the middle stage

301 (Fig. 6d). There were few neurons with significant correlations between the firing rate and licking performance

302 (Fig. 6e). In addition, the firing rate of the FL-related LL type (FL-LL) neurons exhibited the largest increase in

303 the LL trial at the three stages, showing a tendency to reduce the average firing rate and increased firing period

304 after the FL across the stages (Extended Data Fig. 6a–c). The proportion of FL-LL neurons during the learning

305 processes displayed similar changes as observed in the FL-HR neurons (Extended Data Fig. 6d). These results

306 indicate that the aDLS contained neurons showing long-lasting, increased activity after the reward in the specific

307 correct combination of the stimulus and response (HR or LL trial), and that the extent of firing activity and length

308 of firing period in the response appeared to be gradually reduced with no correlation between the proportion of

309 neurons and the progress of the learning stages.

310 In the pVLS, FL-HR neurons showed a sustained elevation in the firing rate after receiving a reward in

311 the HR trial compared to that in the LR or HL trial throughout the three stages (Fig. 6f, top rows). A similar

312 elevation in the firing rate was also observed in the LL trial throughout the stages (Fig. 6f, top rows). The auROC

313 values revealed a significant difference of the LR or HL trial, but not the LL trial, from the HR trial throughout the

314 stages (Fig. 6f, middle rows). The average firing rate did not show significant changes among the three stages

315 (Fig. 6g), and the increased firing period was also similar across the stages (Fig. 6h). The proportion of FL-HR
316 neurons increased at the late stage compared to the early or middle stage (Fig. 6i). The firing rate showed almost
317 no significant correlation to the licking performance (Fig. 6j). In addition, FL-LL neurons exhibited similar firing
318 patterns and changes in the proportion of neurons across the stages (Extended Data Fig. 6f–j). Therefore, the
319 pVLS included neurons representing the long-lasting increased activity after the reward in the two kinds of correct
320 responses (HR and LL trials) with the increased number of these neurons at the late stage of learning.
321
322 **Neurons in the pVLS, but not in the aDLS, exhibit firing patterns related to the beginning and ending of**
323 **the behavior**
324 The dorsal striatum plays a key role in the acquisition and execution of behaviors based on the stimulus-response
325 association, and the association is accompanied by a transient activation of neuronal firing immediately after the
326 stimulus presentation or action in trials, which is considered to represent the beginning and ending of a learned
327 behavior[37-39]. We first examined whether the firing activity of CO-related HR type (CO-HR) or CO-related LL
328 type (CO-LL) neurons corresponds to the beginning of the behavior in the auditory discrimination task. In the
329 aDLS, the firing rate of CO-HR or CO-LL neurons showed a significant difference at some time points in each
330 trial compared to those in any of the other three trials, with a significant difference of auROC values between the
331 trials (Extended Data Fig. 7a,c). However, the transiently increased activity did not emerge at the time points
332 corresponding to the beginning of the behavior. In contrast, CO-HR neurons in the pVLS showed a transient
333 increase in the firing rate immediately after the CO, and the activity appeared at the middle and late stages (Fig.
334 7a). The transient activation was observed not only in the HR trial but also in the other three trials, being
335 consistent with the results of a previous report[40]. The increased firing rate showed a slight but notable difference
336 between the HR and LL trials with a significant difference in the auROC values between the two trials (Fig. 7a),
337 suggesting that different combinations between the stimulus and response may affect the level of firing activity.
338 Differences in the average firing rate between the two kinds of trial was observed at the middle and late stages
339 (Fig. 7b). The proportion of CO-HR neurons increased at the middle and late stages compared to the early stage
340 (Fig. 7c). In addition, the firing rate of CO-LL neurons exhibited patterns similar to those observed in the CO-HR
341 neurons (Extended Data Fig. 7e). The data indicate that subpopulations of pVLS neurons show firing activity
342 related to the beginning of the behavior, with a rising number of neurons throughout discrimination learning.
343 Next, we examined the firing activity of CR-related HR type (CR-HR) or CR-related LL type (CR-LL)
344 neurons, corresponding to the ending of a behavior in auditory discrimination. In the aDLS, the firing rate of these

345 neurons did not exhibit the transient increase related to the ending of the behavior, instead, there was a modest
346 increase before the lever press (Extended Data Fig. 7b,d). In contrast, CR-HR neurons in the pVLS were
347 transiently activated immediately after the lever press in the four kinds of trial at the middle and late stages, and
348 the rate appeared to gradually decline toward the RS presentation (Fig. 7d). The averaged firing rate was similar
349 between the HR and LL trials though the stages (Fig. 7e). The proportion of the CR-HR neurons increased at the
350 middle and late stages compared to the early stage (Fig. 7f). In addition, the rate of CR-LL neurons displayed
351 similar firing patterns to those observed in the CR-HR neurons (Extended Data Fig. 7f). Thus, subpopulations of
352 pVLS neurons also represent the activity related to the ending of the behavior in auditory discrimination,
353 increasing the neuron number throughout the learning stages in a similar pattern to neurons relevant to the
354 beginning of the behavior in the task.
355

356 **Discussion**

357 Our findings demonstrate that the aDLS and pVLS are required for the new learning of auditory discrimination at
358 different stages of the acquisition phase, and that the DMS is not necessary for the acquisition of discrimination,
359 although it appears to contribute to the execution of actions, in particular to the regulation of response time. These
360 results not only support some recent studies reporting no requirement of the DMS in the procedural learning[20,41,42],
361 but also reveal important roles of two different subregions in the lateral striatum in the external cue-dependent
362 decision-making. We propose that aDLS and pVLS neurons act to integrate the new learning of auditory
363 discrimination in spatiotemporally and functionally distinct manners, showing a remarkable difference from the
364 prior learning model that proposes the functional dominance changes from the DMS to DLS subregions during
365 procedural learning[12-16].

366 Our ^{18}F-FDG-PET imaging study demonstrated that the brain activity in the aDLS reaches a peak at the
367 middle stage of the acquisition phase of auditory discrimination (Fig. 2i). The results of excitotoxic lesion
368 experiment of striatal subregions suggest that the aDLS functions to enhance the acquisition of discrimination
369 through the promotion of the stimulus-response association and the suppression of the response-outcome
370 association (Fig. 3j), and transient inhibition experiment of neuronal activity confirmed this aDLS function at the
371 middle stage of the learning (Fig. 4e,f). In addition, we found subpopulations of aDLS neurons (RS-related
372 neurons) which represent the behavioral outcome and differentiate the correct (HR or LL) and incorrect (LR or
373 HL) choices (Fig. 5d). The distinct responses of these neurons may explain the mechanism by which the aDLS

regulates the stimulus-response and response-outcome associations through distinct neural circuits leading to a differential control of the two associations. Alternatively, the responses mainly promote the stimulus-response association, thereby secondarily inhibiting the response-outcome association. Indeed, some previous studies have suggested that an action based on either the stimulus-response association or response-outcome association is generated as a result of competition between different striatal subregions[43]. We also found other subpopulations of aDLS neurons (FL-related neurons) showing long-lasting increased activity after obtaining reward in response to a specific combination between the stimulus and response (HR or LL trial) (Fig. 6a). This activity may function as the feedback signal to promote the stimulus and response association, resulting in the enhancement of discrimination learning. The proportion of neurons related to the behavioral outcome showed a gradual reduction through the progress of auditory discrimination (Fig. 5e and Extended Data Fig. 5c), and the extent of the firing activity and the length of the firing period of neurons showing sustained activation after reward were also decreased at the late stage of learning (Fig. 6b,c). The learning-dependent changes in the properties of these subpopulations in the aDLS may explain the mechanism underlying the earlier acquisition process of the auditory discrimination.

The ^{18}F-FDG-PET imaging indicated that the pVLS activity was gradually elevated through the progress of auditory discrimination, showing the maximal value at the late stage (Fig. 2j). The lesion experiment suggested that the pVLS drives the formation of discrimination learning via the selective progression of the stimulus-response association (Fig. 3k), and the transient inhibition experiment ascertained the pVLS function at the late stage of learning (Fig. 4g,h). Previous studies have shown that the stimulus-response association is encoded in the DLS or VLS as the transient neuronal activity representing the beginning and ending of a learned behavior[37-39,44]. A recent study reported that the VLS is causally related to learned performance based on the auditory stimulus, while the DLS shows a weaker relationship to the behavior[22]. In the present study, we found subpopulations of pVLS neurons that represent the beginning and ending of a behavior (CO- and CR-related neurons, respectively) (Fig. 7a,d), showing an increased number of neurons along with the progress of discrimination learning (Fig. 7c,f). These data suggest that pVLS neurons contribute to the formation of the stimulus-response association through the learning processes. In addition, we found another subpopulation of pVLS neurons (FL-related neurons) that are continuously activated after the reward outcome in response to two kinds of correct choices (HR and LL trials) (Fig. 6f), and the proportion of neurons was increased at the late stage (Fig. 6i). This activity may act as a feedback signal to consolidate the stimulus and response association, leading to the maintenance of associative memory at the late stage during the acquisition phase of learning.

In the present study, we identified neurons with four kinds of event-related activity in the aDLS and pVLS, and classified them into the HR and LL type neurons in which their firing activity showed a significant increase in the HR and LL trials, respectively, as compared to the baseline (Fig. 5–7, and Extended Data Fig. 6,7). Parts of these two types of neurons with the respective event-related activity overlapped, and the numbers of overlapping neurons varied between striatal subregions (Extended Data Figure 8a,b). In the pVLS, the overlapping neurons for the same event-related activities were 35.1%, 45.2%, and 66.9% in the CO, CR, and FL, respectively. In contrast, the aDLS had lower overlaps of 2.6% and 22.7% in the RS and FL, respectively (Extended Data Figure 8a,b). Thus, distinct neurons in the aDLS tend to have properties of a single trial type while the same neurons in the pVLS seem to possess properties of both types. One possible explanation for the diversity in the proportion of overlapping neurons between the two subregions may be the difference of input patterns into striatal neurons or plastic changes in functional connectivity during learning processes. The detailed mechanism by which aDLS and pVLS neurons react to the events in a different manner should be investigated in the future.

Anatomically, striatal subregions are known to form heterogeneous cortico-basal ganglia loops in mice[2,45,46]. We examined the neural circuits linked to the aDLS and pVLS in rats by using bidirectional tracers. The results showed that two striatal subregions receive innervations from different subregions in the cerebral cortex, intralaminar thalamus, and substantia nigra pars compacta, and send projections to different subregions in the basal ganglia nuclei, including the globus pallidus, entopeduncular nucleus, and substantia nigra pars reticulata (Extended Data Fig. 9), confirming that the aDLS and pVLS form the heterogenous cortico-basal ganglia loops in the rat brain. These data suggest that the aDLS and pVLS mediate the acquisition of auditory discrimination via the parallel loops, and cooperate to achieve neural processing related to the dual regulation of the stimulus-response and response-outcome associations. Further studies are needed to elucidate the mechanism by which the two kinds of loops are processed separately and integrated the new learning of discrimination.

Materials and Methods

Animals

Animal care and handling procedures were carried out in accordance with the guidelines established by RIKEN Center for Biosystems Dynamics Research, Fukushima Medical University, and Osaka City University. All procedures were approved by their Institutional Animal Care and Use Committees. All efforts were made to

433 minimize the number of animals used and their suffering throughout the course of the experiments. Male Long-
434 Evans rats (8–13 weeks old) were used for the present study. The rats were maintained at 22 ± 2 °C and 60%
435 humidity in a 12-h light/12-h dark cycle, and food and water were continuously available unless stated otherwise.
436
437 **Surgery**
438 The rats underwent a stereotaxic surgery under isoflurane anesthesia (1–5%). For excitotoxic lesion of striatal
439 neurons, IBO was dissolved in PBS (0.8 mg/mL, Wako Pure Chemical), and IBO solution (0.3 μL per site) was
440 injected bilaterally with the coordinates (mm) from bregma and dura for the aDLS (AP + 1.6, ML ± 3.3, DV -
441 3.9), pVLS (AP - 1.6, ML ± 4.6, and DV - 5.6), or DMS (AP - 1.6, ML ± 1.4, and DV - 4.8) according to the atlas
442 of rat brain[47]. Injection was performed through a glass pipette (diameter: 60 μm) at a constant velocity of 0.1
443 mL/min with the microinfusion pump (EICOM). For transient inactivation of striatal neurons, two guide cannulae
444 (length: 10 mm, outer diameter: 26 Gauge) were bilaterally placed on the skull and fixed using four screws and
445 dental cement. The chip of the cannula was placed at 1 mm above the target coordinates. A solution containing
446 MUS (Sigma-Aldrich) in saline (0.1 μg/μl, 0.25 μL per site) was injected into the target coordinates through the
447 internal cannula (outer diameter: 30 Gauge) at a constant velocity of 0.25 μL /min. For anterograde and retrograde
448 tracing, a solution containing cholera toxin subunit B (CTb; 1.0 mg/mL) conjugated to Alexa 488 or Alexa 555
449 (Invitrogen) as a bidirectional tracer was injected into the aDLS and pVLS (0.3 μL/site), respectively. The
450 coordinates for the injection into striatal subregions were the same as described above. For the
451 electrophysiological experiments, surgery was performed as described previously[48]. A 64-channel silicon probe
452 (Buzsaki64spl; NeuroNexus) attaching to a 3D-printed microdrive with a movable screw was chronically
453 implanted with the coordinates (mm) from the bregma and dura into the left aDLS (AP + 1.6, ML - 3.3, and DV -
454 2.5) and pVLS (AP - 1.6, ML - 4.6, and DV - 3.6). A faraday cage composed of copper mesh was secured on the
455 skull with dental cement to reduce electrical noise and avoid damage to the implants. The rats were allowed to
456 recover for 1 week after the surgeries.
457
458 **Behavioral analysis**
459 Behavioral tasks were conducted in operant conditioning chambers (30.5 × 24.1 × 29.2 cm; ENV-007CT; MED
460 Associates) equipped with two retractable levers on both sides of a reward port in the front panel (ENV-203M-45,
461 ENV-652AM, and ENV-201A; MED Associates). A multi-tone generator (ENV-223; MED Associates) and room
462 light were mounted on the top of the center and corner panels on the rear side, respectively. For the

463 electrophysical experiment, a 3D printed reward port was used to detect the licking and to prevent contact with the
464 head attachment and front panel. The behavioral experiment in intact rats was performed using grain pellets
465 (F0021, Bioserv) as a food reward. To prevent spillover of radioactivity into the temporal cortex as a consequence
466 of excessive ^{18}F-FDG accumulation into the muscles by their activity to eat the pellets[49,50], the PET imaging
467 experiments were carried out using 0.02% saccharine solution (B0131, Tokyo Chemical Industry) as a liquid
468 reward. The electrophysiological experiment was also carried out using the saccharine solution. The
469 pharmacological experiment was performed using the pellets.

470 The rats were subjected to dietary restrictions of 12 g/day (CE-2, CLEA Japan) and water access was
471 limited to 6 to 9 h in accordance with the reward used in the experiments for 3 days before the beginning of the
472 training schedule, and the body weights of the rats were maintained at ~85% of their normal weights. The training
473 schedule consisted of four continuous steps. In the first step (magazine training), the rats were habituated to the
474 operant conditioning chambers, and the reward was presented with the click every 20 s. Each daily session was
475 started by illumination of the room light, and continued for 30 min. The sessions were conducted for 3
476 consecutive days. In the second step (shaping), only one lever on the left or right side was inserted. When the rats
477 pressed the lever, they earned a reward accompanied by a click. Each daily session was started by illumination of
478 the light, and lasted 30 min or until the criteria, in which the rats received 90 rewards. When the number of lever
479 presses on either side reached the criteria, the lever was inserted in the opposite side on the next day. When the
480 rats achieved the criteria on both sides for 2 or 3 days, they moved to the following step. In the third step (single
481 lever press task), each trial was started by illumination of the light and insertion of one lever on either the left or
482 right side in a pseudorandom manner with equal frequencies on both sides for every set of four trials. When the
483 rats pressed the lever, the reward was presented with the sound, and the lever was immediately retracted. The
484 room light turned off 4 s after the lever press. When they did not press the lever within 10 s after the start, the trial
485 was terminated when the light turned off. The trial was repeated every 20 s. Each daily session continued for 30
486 min, and the sessions were conducted for 4 consecutive days. The number of lever press in each session was
487 counted. In the final step (two-alternative auditory discrimination task), each trial was started by the presentation
488 of a high (10 kHz) or low (2 kHz) frequency tone as an instruction cue in a pseudorandom manner. After three
489 seconds, the room light was illuminated, and two retractable levers were inserted at the same time. The rats were
490 required to press the left or right levers in response to the low or high tone cues, respectively. When the rats
491 pressed the correct lever, the reward was presented with the sound, and the levers were immediately retracted.
492 Correct response allowed the rats to access the reward: the retractable spout delivering water was inserted for 1 s

493 for the PET imaging experiment; a pellet was delivered by a food dispenser for the pharmacological experiment;
494 and three drops of water were delivered through a solenoid valve for the electrophysiological experiment. The
495 room light turned off 4 s after the correct response. On the other hand, error response resulted in the retraction of
496 levers and the turning off of the room light. No response within 10 s after the lever insertion was recorded as an
497 omission response. The success rate in each session was calculated by dividing the number of correct response
498 trials with the number of lever press trials minus the number of omission trials. The trial was repeated every 20 s.
499 Each daily session continued for 60 min, and the sessions were conducted for 14 or 24 days according to the
500 experiments.

501 To assess the behavioral strategies in the auditory discrimination task, a trial-by-trial analysis was
502 conducted; the WSW strategy was defined as a correct response, in which, after a correct response during the
503 previous trial, the rats press the opposite lever during the current trial in response to the shift of the tone
504 instruction cue; the LSL strategy was defined as an error response, in which, after an error response during the
505 previous trial, the rats press the opposite lever during the current trial despite the shift of the instruction cue. The
506 proportion of both strategies was calculated by dividing the number of each strategy with the number of lever
507 press trials minus the number of omission trials.

508 For the transient inactivation experiment, MUS injections were bilaterally performed 20 min before start
509 of the session. For habituation, all rats were injected with saline bilaterally into the corresponding striatal
510 subregions on the last day (Day 4) of the single lever press task. During the auditory discrimination task, the first
511 injection was conducted on Day 2 of the task. The second and third injections were conducted the next day, after
512 the success rate had exceeded 60% and 80%, respectively. Each session continued for 15 min.

513 For the electrophysiological experiments, in the auditory discrimination task, a delay period (0.5 ± 0.2 s)
514 was added between the lever press and reward sound to distinguish the motor- and reward-related responses of
515 neurons. When the rats pressed the lever, the lever was immediately retracted. After the delay period, the reward
516 was presented with a sound. The room light turned off 4 s after the lever press. The trial was repeated every 20 s,
517 and each daily session continued for 60 min.
518

519 **PET imaging analysis**

520 An ^{18}F-FDG-PET scan was performed with a microPET Focus220 (Siemens Medical Solutions, Knoxville)
521 designed for high-resolution imaging of small animals (spatial resolution of 1.4 mm in full width at half maximum
522 at the center of the field of view) as described previously[26]. Serial ^{18}F-FDG-PET scans were performed on the last

523 day of the single lever press task and on Days 2, 6, 10, and 24 of the auditory discrimination task. Under the
524 awake condition, a solution of ^{18}F-FDG (ca. 74 MBq/0.4 mL) was intravenously injected just before the beginning
525 of each behavioral session through an indwelling catheter attached to the tail. After the 30-min session, the rats
526 were returned to their home cage, and then a 30-min static PET scan was performed under anesthesia with 2–2.5%
527 isoflurane 55-min after the ^{18}F-FDG injection. During the PET scan, the body temperature of the anesthetized rats
528 was maintained at 37°C using a small animal warmer (BWT-100A, Bio Research Center). The acquired emission
529 data were sorted into a single sinogram and reconstructed by a standard 2D filtered back projection (FBP) with a
530 ramp filter and cutoff frequency at 0.5 cycles per pixel or by a statistical maximum a *posteriori* probability
531 algorithm (MAP), 12 iterations with point spread function effect.
532 Voxel-based analysis of PET images was conducted according to the method described previously[26].
533 Briefly, individual MAP-reconstructed FDG (MAP-FDG) images were aligned to a FDG template image of Long
534 Evans rats using PMOD software package (version 3.2, PMOD Technologies), and then the aligned images were
535 transformed into the space of a T2 weighted MRI templates for the rats (https://www.nitrc.org/projects/tpm_rat/).
536 The transformation parameters obtained from individual MAP-FDG images were used to match the individual
537 FBP-reconstructed FDG (FBP-FDG) images with the MRI templates. The voxel size of the template was
538 magnified by 10 to a size similar to that of a human brain. All FBP-FDG images were resampled with a voxel size
539 of 1.2 × 1.2 × 1.2 mm and spatially smoothed through an isotropic Gaussian kernel (6-mm FWHM). Voxel-based
540 statistical analysis was assessed by using the Statistical Parametric Mapping software (version 8;
541 https://www.fil.ion.ucl.ac.uk/spm/). The voxel of interest (VOI) was determined as voxels with more than 85% of
542 the maximum T-value within activated areas obtained by voxel-based statistical comparison of FBP-FDG images.
543 The average regional ^{18}F-FDG uptake was calculated on the basis of the values of the VOI in FBP-FDG images.
544
545 **Electrophysiological recording**
546 Extracellular multi-unit recording was conducted at a sampling rate of 20 kHz from the striatum of freely moving
547 rats by using a 512-ch acquisition board (Open Ephys) via 64-ch recording headstages (C3325; Intan
548 Technologies). The tips of the probe were lowered by turning the screw (> 140 μm/day) until it reached the target
549 region (the aDLS and/or pVLS) during the single lever press task. The tips were sometimes lowered by the same
550 procedure (140 to 280 μm/day) after the daily recording session to improve cell yield.
551 Spike sorting was performed automatically by the Kilosort software (https://github.com/cortex-
552 lab/KiloSort). Clear noise clusters were removed in the first manual sorting using the phy GUI

(https://github.com/cortex-lab/phy). The remaining clusters were then re-clustered by using the Klusta Kwik (https://klusta.readthedocs.io/en/latest/), and the clusters were adjusted in the second manual sorting by using the phy GUI again. The sorted clusters satisfying all the following criteria were used for further analysis: L-ratio < 0.05, isolation distance > 15, and ISI index < 0.2[51,52]. Well-isolated single neurons (n = 1485; aDLS, n = 1062; pVLS, n = 423) were classified as putative medium spiny neurons (MSNs), fast spiking interneurons (FSIs), tonically active neurons (TANs), and unclassified interneurons (UIs). Putative MSNs and TANs were separated from FSIs and UIs by trough-to-peak duration of mean spike waveform (> 0.6 ms) according to previous studies, with slight modifications[53,54]. Putative FSIs were then separated from UIs by utilizing the proportion of time associated with long inter-spike intervals. This was achieved by summing the inter-spike intervals longer than 2 s and subsequently dividing the resulting sum by the total recording time ($Prop_{ISIs} > 2$ s)[55]. Neurons with values of $Prop_{ISIs} > 2$ s less than 0.4 were classified as putative FSIs, while neurons with $Prop_{ISIs} > 2$ s greater than 0.4 were classified as putative UIs. The putative MSNs and TANs were separated by measuring the post-spike suppression[55]. We measured the length of suppressed time during which the firing rate of a neuron was suppressed following a spike by counting the number of 1-ms bins in its autocorrelation function until the rate of the neuron was equal to or greater than its averaged rate over the 600-ms to 900-ms autocorrelation bins. Putative TANs were separated by post-spike suppression of less than 40 ms, and the remaining neurons were classified as putative MSNs. Because the numbers of FSIs, TANs, and UIs were low (67 out of 1485, 5.1%), they were excluded from further analysis.

To divide the three stages during the acquisition phase, the sigmoidal function was fitted using the following four-parameter logistic equation:

$$f_{(x)} = \frac{1 - \gamma - (1 - \lambda)}{1 + \exp\left(-\beta(x - \alpha)\right)} + \gamma$$

Where α is the inflection point, β is a variable for slope, and λ and γ are parameters related to the lower asymptote (γ) and upper asymptote (λ) of the learning curve. The function was used to evaluate the day when the success rate reached 20% ($t_{20\%}$) or 90% ($t_{90\%}$) of the maximum rate change defined by the difference between the γ and λ. Three stages were defined according to the following criteria: early stage (up to 3 days before the middle stage); middle stage (3 days including before and after the day of $t_{20\%}$); and late stage (3 days including the day of $t_{90\%}$ and before). One rat learned the discrimination faster, and the success rate reached $t_{90\%}$ 2 days after the day of $t_{20\%}$. One day before the day of $t_{90\%}$ was assigned to the late stage. For the following analysis, spike data in each striatal subregion were separated into early, middle, and late stages (*n* = 295, 364 and 360, respectively, for aDLS; and *n*

582 = 87, 110 and 190, respectively, for pVLS).

583 To determine the neurons showing significant changes in firing rate related to the task events,
584 electrophysiological data were grouped into four trial types based on the instruction cue (high or low tone) and the
585 choice (right or left lever); HR, high tone and right lever press; HL, high tone and left lever press; LR, low tone
586 and right lever press; and LL, low tone and left lever press. Neurons with three or more trial data were analyzed.
587 We identified the HR or LL type neurons, detecting significant increases or decreases of the firing rate by
588 applying the Wilcoxon rank-sum test ($p < 0.01$) to trial-by-trial spike number between the baseline period (BL, -
589 1500 to -500 ms or -1100 to -500 ms before presentation of the instruction cue) and any of four peri-event periods
590 (CO, 0 to 1000 ms after the onset of the instruction cue; CR, -700 to +300 ms around the time of lever press; RS, -
591 300 to 300 ms around the time of sound onset instructing the reward delivery; and FL, 0 to 1000 ms after the time
592 of first licking after the CR). Any spikes around the events were averaged in 50-ms bins. The event-related
593 activity was shown as averaged firing rates aligned based on the timing of an event for each trial. The auROC
594 values were calculated by using the spike counts between the HR or LR trials and any of the other three trials.
595 Bins showing significant differences in the auROC data were determined by applying the Wilcoxon rank-sum test
596 ($p < 0.05$) to the distribution of 200 ms bin-by-bin auROC values and 0.5.

597 To compare the number of neurons showing event-related activity among different stages, the number of
598 HR or LL type neurons were calculated by matching the trial number at each stage. Twenty trials were randomly
599 selected for a neuron, and neurons with less than 20 trials were removed from the analysis. The neurons with
600 event-related activity were identified as described above. The proportion (percentage) of neurons relative to the
601 total number of neurons was calculated at each individual stage. The same analysis was repeated 30 times and the
602 distribution of the proportion of neurons with event-related activity at the stage was obtained. The cumulative
603 probability against the proportion was calculated using cumulative distribution function (cdfplot, MATLAB code
604 from Mathworks).

605 Licking responses were detected with an infrared photobeam sensor placed in the 3D printed reward
606 port, and generated timestamps for the onset of each spout contact according to the analog voltage signal. The
607 licking responses corresponding to four kinds of trial in the FL-related neurons were shown as averaged rates (50-
608 ms bins) aligned based on the timing of the CR. To calculate the correlation coefficient between neuronal activity
609 and licking responses, the spikes and licking for 5 s after the FL were counted every trial for each FL-related
610 neuron. Trials in which neurons never responded were removed from both the spike and licking data. The
611 correlation coefficients between the two remaining data were calculated for each neuron (corr, MATLAB code

612 from Mathworks), and neurons showing significant differences were determined by applying the Pearson
613 correlation coefficient ($p < 0.01$).
614
615 **Histology**
616 After the behavioral experiments, the rats were anesthetized with pentobarbital (50 mg/kg body weight) and
617 isoflurane (3%), then transcardialy perfused 4% paraformaldehyde in phosphate-buffer (PB). The brains were
618 post-fixed overnight at 4 °C and cryoprotect with 10, 20, and 30% sucrose in PB. Brain tissues were cut into 30-
619 μm sections by using a cryostat (Leica). For the lesion experiment, cresyl violet staining or immunostaining were
620 performed to define the lesioned areas in the striatum. The sections were stained with a mouse monoclonal anti-
621 NeuN IgG (1:1000 dilution; Millipore Corporate), and then with a goat anti-mouse IgG conjugated with Alexa
622 Fluor 488 (1:400 dilution; Invitrogen). The sections were counterstained with 0.3 μM 4',6-diamidino-2-
623 phenylindole (DAPI; Sigma-Aldrich). For transient inactivation experiment, sections were stained by cresyl violet
624 to visualize the positions of guide cannulae. For the electrophysiological experiments, small lesions were made by
625 applying an electrolytic current (3 μA, 10 s) through top and bottom channels of each shank by using the stimulus
626 isolator (A365, World Precision Instrument). Sections through the striatum were stained with a mouse monoclonal
627 anti-TH IgG (1:1000 dilution; Sigma-Aldrich), and then with a goat anti-mouse IgG conjugated with Alexa Fluor
628 488 (1:400 dilution; Invitrogen). The recording sites were verified by the staining with DAPI and red fluorescent
629 Nissl stain solution (1:200 dilution; Thermo Fisher Scientific). Fluorescence and immunostained signals were
630 visualized under fluorescence microscopes (BZ-X800; Keyence) and confocal microscope (A1R; Nikon).
631
632 **Statistical analyses**
633 Statistical analysis was performed using MATLAB (MathWorks) and SPSS Statistics (Ver. 27, IBM).
634 Comparisons of data between two groups were performed using the Student's *t*-test (paired), the Wilcoxon signed
635 rank test (paired), and the Wilcoxon rank-sum test (unpaired). Comparisons of data among three groups were
636 constructed using a one-way repeated measures ANOVA, two-way repeated measures ANOVA, or the Kruskal-
637 Wallis test (without assuming normal distribution and homogeneity of variances). All statistical tests were two-
638 sided. The Bonferroni post-hoc test and Mann–Whitney U-test with Bonferroni adjustment were carried out as
639 needed to compare means. For boxplots, the data points below the lower (25th percentile) - 1.5 interquartile range
640 (IQR) and above the upper (75th percentile) quartiles + 1.5 IQR were regarded as outliers, and they are not
641 included in the boxplots.

Data availability

All source data underlying the graphs and charts are available in Supplementary Data. All other data (if any) are available upon request. These data will be opened in the Mendeley Data after the manuscript is accepted.

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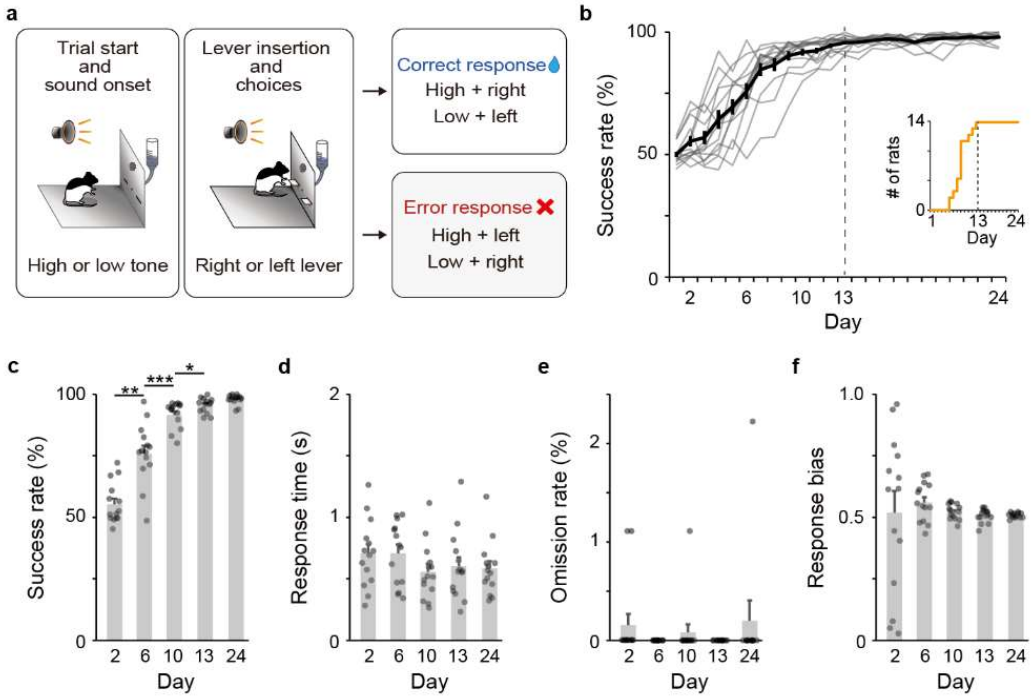
Authors' contributions

S.S., Y.C., and K.K. designed the study. S.S. performed the small-animal neuroimaging, multi-unit recordings, tracing studies, and pharmacological experiments with technical support from all authors; T.O., D.H., Y.W., H.O., K.H., and Y.C. assisted with the neuroimaging studies. K.N. performed the tracing study, and N.S. assisted with the immunostaining. H.M., T.K., and K.M. assisted with the multi-unit extracellular recordings. S.S. and K.K.

wrote the manuscript with assistance from all coauthors.



783 **Figure 1. Behavioral performance of two-alternative auditory discrimination learning.**

784 **a**, Schematic diagram of the auditory discrimination task. Each trial started by presentation of a tone instruction cue
785 with the high (10 kHz) or low (2 kHz) frequency. Three seconds later, the room light was illuminated, and two
786 retractable levers were inserted at the same time. The rats were required to press the right and left levers in response
787 to the high and low tones, respectively. **b**, Learning curve of the auditory discrimination in intact rats (n = 14). Inset
788 indicates the cumulative curve of number achieved the success rate of more than 90%. **c**, Success rate at Days 2, 6,
789 10, 13, and 24 (one-way repeated ANOVA, $F[1.897,24.659] = 106.706$, $p = 1.1 \times 10^{-12}$, *post hoc* Bonferroni test,
790 Day 2 vs. Day 6, $p = 0.001$, Day 6 vs. Day 10, $p = 3.6 \times 10^{-4}$, Day 10 vs. Day 13, $p = 0.040$, and Day 13 vs. Day 24,
791 $p = 0.080$). **d**, Response time (one-way repeated ANOVA, $F[2.203,28.642] = 2.386$, $p = 0.105$). **e**, Omission rate
792 ($F[4,52] = 0.699$, $p = 0.596$). **f**, Response bias (one-way repeated ANOVA, $F[1.129,14.674] = 0.250$, $p = 0.653$).
793 Data are indicated as the mean ± s.e.m., and individual data are overlaid. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.
794

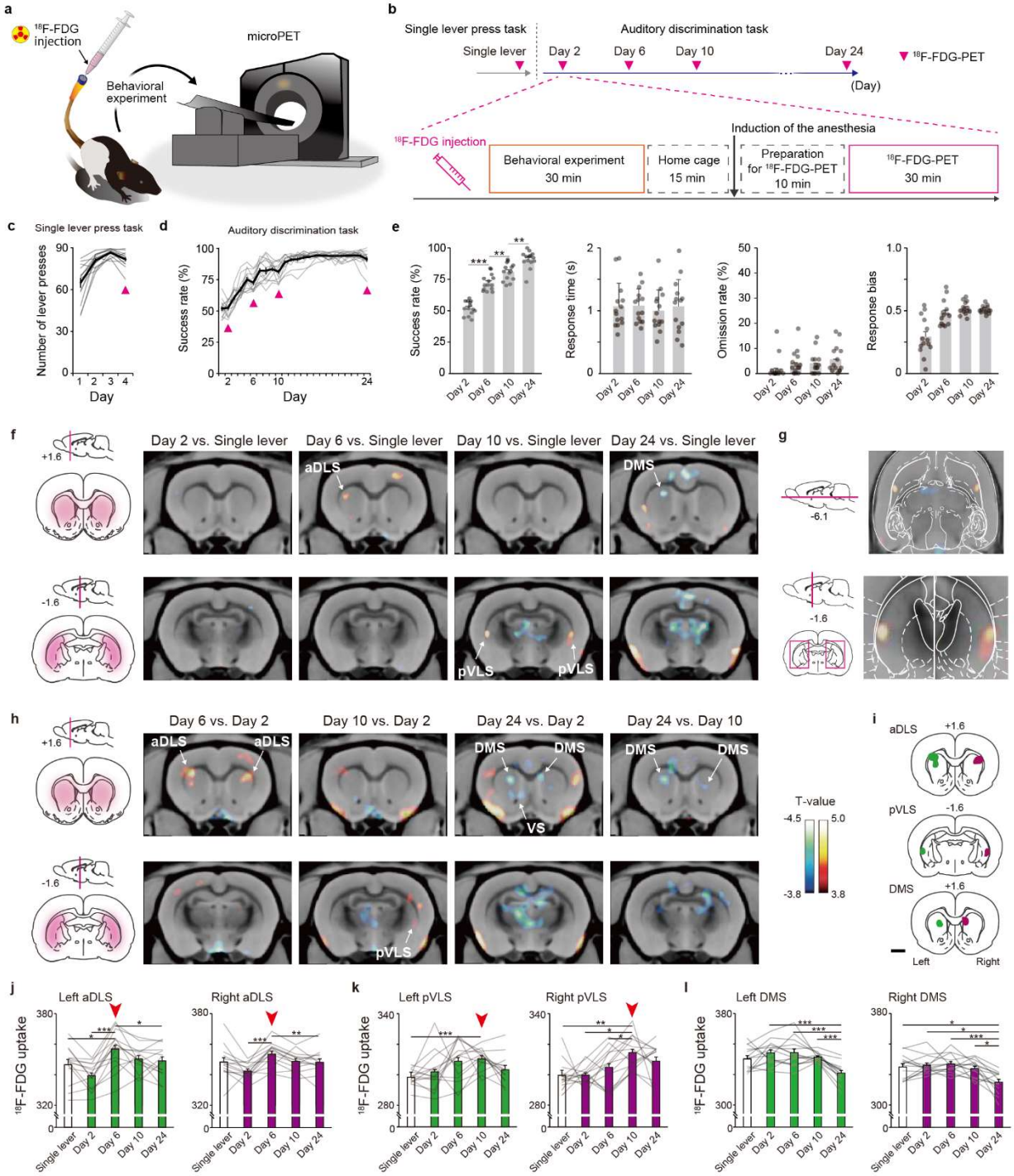


Figure 2. Dissociable brain activity patterns during auditory discrimination learning among three striatal subregions. a, Schematic illustration of an awake rat received intravenously ^{18}F-FDG injection through an indwelling catheter attached to the tail. The rat was conducted for the behavioral experiment and then used for microPET imaging. **b**, Schedule for the behavioral training and ^{18}F-FDG-PET scan. After ^{18}F-FDG injection, rats (n = 14 rats) were subjected to the behavioral experiment (30 min) and returned to the home cage (15 min). Ten min before the PET scan started, the rats were anesthetized and then the scan was started (30 min). **c**,**d**, Learning curves of the single lever press task (**c**) and auditory discrimination task (**d**). Arrowheads indicate the PET scan days. **e**,

Behavioral performance on the scan days during the auditory discrimination. Success rate (one-way repeated ANOVA, $F[3,39] = 125.012$, $p = 4.8 \times 10^{-20}$, *post hoc* Bonferroni test; Day 2 vs. Day 6, $p = 5.0 \times 10^{-7}$; Day 6 vs. Day 10, $p = 0.001$; Day 10 vs. Day 24, $p = 0.003$), response time (one-way repeated ANOVA, $F[3,39] = 0.156$, $p = 0.926$), omission rate (one-way repeated ANOVA, $F[3,39] = 0.156$, $p = 0.215$), and response bias (one-way repeated ANOVA, $F[1.779,23.130] = 17.734$, $p = 3.6 \times 10^{-5}$) are shown. **f**, Representative images of coronal sections that compared the brain activity in the single lever press task with the activity on Day 2, 6, 10, or 24 in the discrimination task. Left panels show schematic illustrations of striatal subregions. **g**, Horizontal (top) and coronal (bottom) images of striatal activation areas shown on Day 10 vs. single lever in (**f**). **h**, Representative images of coronal section that compared the brain activity on Day 2 with that on Day 6, 10, or 24 in the discrimination task. Color bars indicate the T-values, and a value of 3.8 was used as the threshold corresponding to the uncorrected threshold ($p < 0.001$). **i**, Schematic pictures showing voxel of interests for the aDLS, pVLS, and DMS (green, left hemisphere; and purple, right hemisphere). **j–l**, Regional ^{18}F-FDG uptakes in the aDLS (**j**, one-way repeated ANOVA, left aDLS, $F[4,52] = 10.322$, $p = 3.0 \times 10^{-6}$, *post hoc* Bonferroni test; single lever vs. Day 6, $p = 0.016$; Day 2 vs. Day 6, $p = 6.5 \times 10^{-5}$; Day 2 vs. Day 10, $p = 0.019$; Day 2 vs. Day 24, $p = 0.017$; Day 6 vs. Day 24, $p = 0.041$; right aDLS, $F[4,52] = 7.462$, $p = 7.9 \times 10^{-5}$, *post hoc* Bonferroni test, Day 2 vs. Day 6, $p = 2.9 \times 10^{-4}$; Day 2 vs. Day 10, $p = 0.036$; Day 6 vs. Day 24, $p = 0.005$), pVLS (**k**, one-way repeated ANOVA, left pVLS, $F[2.368,30.784] = 4.152$, $p = 0.020$, *post hoc* Bonferroni test; single lever vs. Day 10, $p = 1.8 \times 10^{-4}$; right pVLS, $F[4,52] = 5.995$, $p = 4.8 \times 10^{-4}$, *post hoc* Bonferroni test, single lever vs. Day 10, $p = 0.001$; Day 2 vs. Day 10, $p = 0.011$; Day 6 vs. Day 10, $p = 0.032$), and DMS (**l**, one-way repeated ANOVA, left DMS, $F[4,52] = 12.8362$, $p = 2.4 \times 10^{-7}$, *post hoc* Bonferroni test; Day 2 vs. Day 24, $p = 1.8 \times 10^{-5}$; Day 6 vs. Day 24, $p = 4.8 \times 10^{-4}$; Day 10 vs. Day 24, $p = 5.5 \times 10^{-5}$; right DMS, $F[4,52] = 10.717$, $p = 2.0 \times 10^{-6}$, *post hoc* Bonferroni test; single lever vs. Day 24, $p = 0.036$; Day 2 vs. Day 24, $p = 0.011$; Day 6 vs. Day 24, $p = 2.6 \times 10^{-4}$; Day 10 vs. Day 24, $p = 0.011$). Arrowheads indicate the day with the most activations throughout the learning process. Data are indicated as the mean ± s.e.m., and individual data are overlaid. The anteroposterior coordinates from bregma (mm) are shown (**f–i**). Scale bar; 2 mm (**i**). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

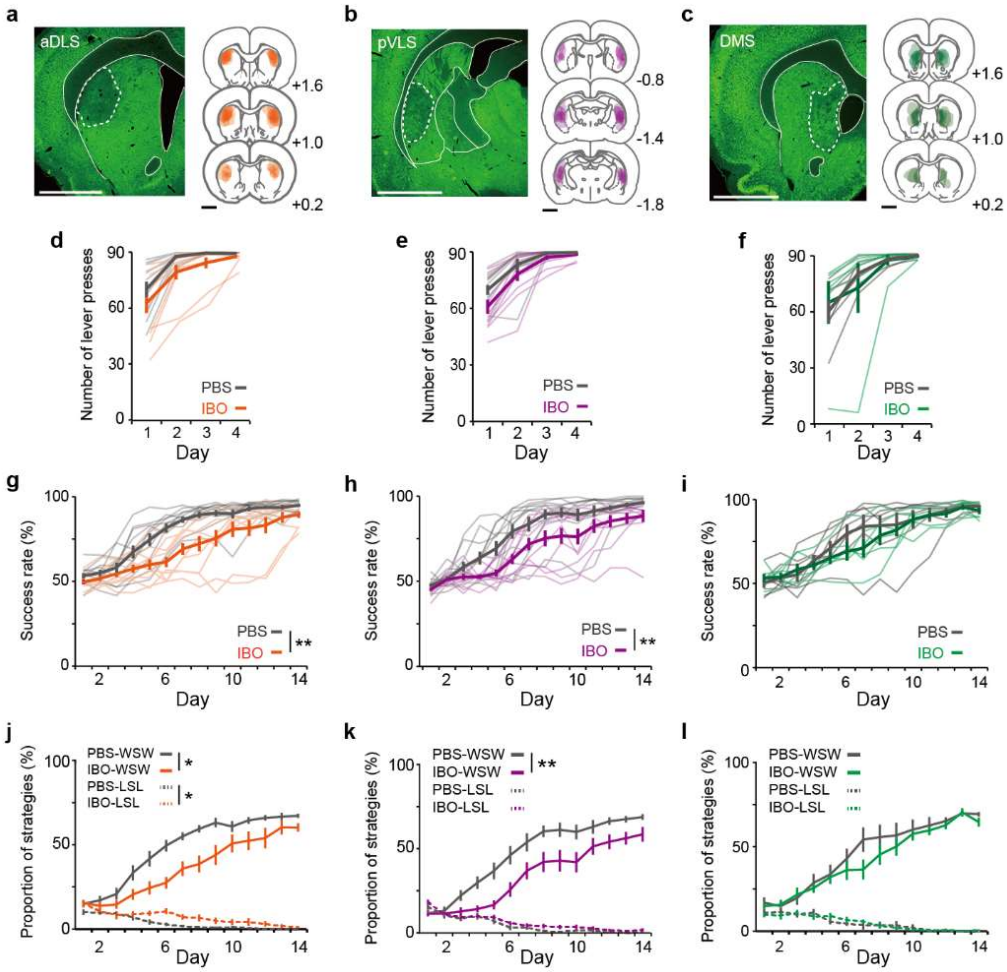


Figure 3. **Impacts of excitotoxic lesion of striatal subregions on the acquisition of auditory discrimination.** Rats were given intracranial injection of PBS or IBO solution into the aDLS (n = 8 for each injection), pVLS (n = 8 for each injection), or DMS (n = 7 for PBS injection, and n = 6 for IBO injection) before start of the single lever press task. **a–c**, Representative images and individual schematic illustrations showing the lesioned area in the aDLS (**a**), pVLS (**b**), or DMS (**c**). **d–f**, Learning curves during the single lever press task in the groups injected into the aDLS **(d,** two-way repeated ANOVA, group, $F[1,14] = 1.275$, $p = 0.278$, day, $F[1.512,21.171] = 26.281$, $p = 7.0 \times 10^{-6}$, group × day, $F[1.512,21.171] = 0.834$, $p = 0.418$), pVLS (**e**, two-way repeated ANOVA, group, $F[1,14] = 2.542$, $p = 0.133$, day, $F[1.547,21.658] = 40.183$, $p = 2.2 \times 10^{-7}$, group × day, $F[1.547,21.658] = 0.953$, $p = 0.380$), or DMS (**f**, two-way repeated ANOVA, group, $F[1,11] = 0.025$, $p = 0.876$, day, $F[1.205,13.252] = 14.818$, $p = 0.001$, group × day, $F[1.205,13.252] = 0.610$, $p = 0.478$). **g–i**, Learning curves during the auditory discrimination task in the groups injected into the aDLS (**g**, two-way repeated ANOVA, group, $F[1,14] = 11.578$, $p = 0.004$, day, $F[2.805,39.274] = 60.733$, $p = 1.8 \times 10^{-14}$, group × day, $F[2.805,39.274] = 3.863$, $p = 0.018$), pVLS (**h**, two-way repeated ANOVA, group, $F[1,14] = 7.221$, $p = 0.018$, day, $F[3.646,51.041] = 51.794$, $p = 9.2 \times 10^{-17}$, group × day, $F[3.646,51.041] = 1.612$, $p = 0.190$), or DMS (**i**, two-way repeated ANOVA, group, $F[1,11] = 0.469$, $p = 0.507$, day,

$F[2.677,29.452] = 48.860$, $p = 3.9 \times 10^{-11}$, group × day, $F[2.677,29.452] = 1.094$, $p = 0.362$). **j–l**, Changes in the proportion of the WSW or LSL strategy through the discrimination learning in the groups treated into the aDLS (**j**, two-way repeated ANOVA, group, $F[1,14] = 13.451$, $p = 0.003$, day, $F[3.048,42.673] = 54.777$, $p = 9.1 \times 10^{-15}$, group × day, $F[3.048,42.673] = 3.288$, $p = 0.029$ for the WSW; group, $F[1,14] = 5.039$, $p = 0.041$, day, $F[3.866,54.119] = 14.578$, $p = 5.2 \times 10^{-8}$, group × day, $F[3.866,54.119] = 2.471$, $p = 0.057$ for the LSL), pVLS (**k**, two-way repeated ANOVA, group, $F[1,14] = 9.251$, $p = 0.009$, day, $F[3.206,44.881] = 47.168$, $p = 2.7 \times 10^{-14}$, group × day, $F[3.206,44.881] = 1.862$, $p = 0.146$ for the WSW; group, $F[1,14] = 0.762$, $p = 0.397$, day, $F[4.773,66.815] = 24.018$, $p = 1.9 \times 10^{-13}$, group × day, $F[4.773,66.815] = 1.338$, $p = 0.260$ for the LSL), or DMS (**l**, two-way repeated ANOVA, group, $F[1,11] = 0.703$, $p = 0.420$, day, $F[3.052,33.568] = 48.424$, $p = 2.3 \times 10^{-12}$, group × day, $F[3.052,33.568] = 0.846$, $p = 0.480$ for the WSW; group, $F[1,11] = 0.002$, $p = 0.965$, day, $F[3.442,37.866] = 13.787$, $p = 1.0 \times 10^{-6}$, group × day, $F[3.442, 37.866] = 0.581$, $p = 0.654$ for the LSL). Data are indicated as the mean ± s.e.m., and individual data are overlaid (except for panels **j–l**). The anteroposterior coordinates from bregma (mm) are shown (**a–c**). Scale bars; 2 mm (**a–c**). *$p < 0.05$ and **$p < 0.01$.

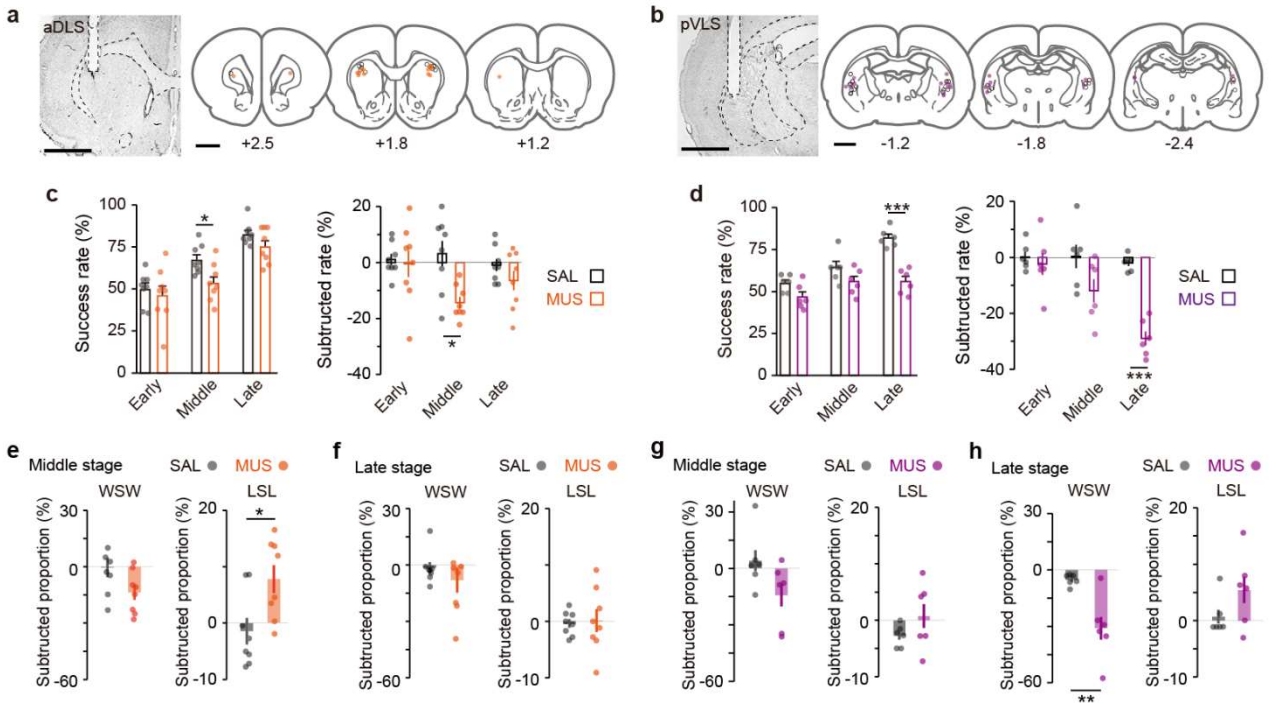


Figure 4. Influence of transient inhibition of striatal subregions at different timings on the performance of auditory discrimination. Rats received intracranial injection of SAL or MUS solution into the aDLS (n = 8 for each injection) or pVLS (n = 6 for SAL injection, and n = 8 for MUS injection). **a**,**b**, Representative images and schematic illustrations showing placement sites of the tip of guide cannula in the aDLS (**a**) or pVLS (**b**). **c**,**d**, Effects of transient striatal inhibition on the performance. For aDLS inhibition (**c**), success rate (left, two-way repeated ANOVA, stage, $F[2,28] = 28.708$, $p = 1.7 \times 10^{-7}$, group, $F[1,14] = 8.840$, $p = 0.010$, stage × group, $F[2,28] = 0.757$, $p = 0.478$; unpaired Student's t-test, early, $t[14] = 0.558$, $p = 0.586$; middle, $t[14] = 2.764$, $p = 0.015$; Welch's t-test, late, $t[12.010] = 1.617$, $p = 0.132$, $p = 0.016$ after a Bonferroni correction method) and subtracted success rate on day N-1 from that on day N (right, two-way repeated ANOVA, stage, $F[2,28] = 1.231$, $p = 0.307$, group, $F[1,14] = 10.797$, $p = 0.005$, stage × group, $F[2,28] = 2.360$, $p = 0.113$; unpaired Student's t-test, early, $t[14] = 0.285$, $p = 0.780$, late, $t[14] = 1.271$, $p = 0.225$, Welch's t-test, middle, $t[9.603] = 3.331$, $p = 0.008$, $p = 0.016$ after a Bonferroni correction method). For pVLS inhibition (**d**), success rate (left, two-way repeated ANOVA, stage, $F[2,20] = 17.642$, $p = 3.8 \times 10^{-5}$, group, $F[1,10] = 43.942$, $p = 5.9 \times 10^{-5}$, stage × group, $F[2,20] = 4.729$, $p = 0.012$; simple main effect, early, $p = 0.055$, middle, $p = 0.109$, late, $p = 4.2 \times 10^{-5}$) and subtracted success rate on day N-1 from that on day N (right, two-way repeated ANOVA, stage, $F[2,20] = 12.1053$, $p = 3.6 \times 10^{-4}$, group, $F[1,10] = 16.310$, $p = 0.002$, stage × group, $F[2,20] = 8.500$, $p = 0.002$; simple main effect, early, $p = 0.595$, middle, $p = 0.085$, late, $p = 3.0 \times 10^{-6}$). **e**,**f**, Subtracted proportion of the WSW or LSL strategy in the groups injected into the aDLS at the middle stage (**e**, unpaired Student's t-test, $t[14] = 2.038$, $p = 0.061$ for the LSL, and $t[14] = 2.714$, $p = 0.017$ for the WSW) and the late stage (**f**, unpaired Student's t-test, $t[14] = 0.898$, $p = 0.384$ for the LSL, and $t[14] = 0.226$, $p = 0.824$ for

874 the WSW). **g**,**h**, Subtracted proportion of the WSW or LSL strategy in the groups injected into the pVLS at the
875 middle stage (**g**, unpaired Student's t-test, $t(10) = 1.924$, $p = 0.083$ for the LSL, and Welch's t-test, $t[6.095] = 1.364$,
876 $p = 0.221$ for the WSW) and the late stage (**h**, unpaired Student's t-test, $t[10] = 3.629$, $p = 0.005$ for the LSL, and
877 $t[10] = 1.577$, $p = 0.146$ for the WSW). Data are indicated as the mean ± s.e.m., and individual data are overlaid.
878 The anteroposterior coordinates from bregma (mm) are shown (**a**,**b**). Scale bars; 2 mm (**a**,**b**). *$p < 0.05$, **$p < 0.01$,
879 and ***$p < 0.001$.

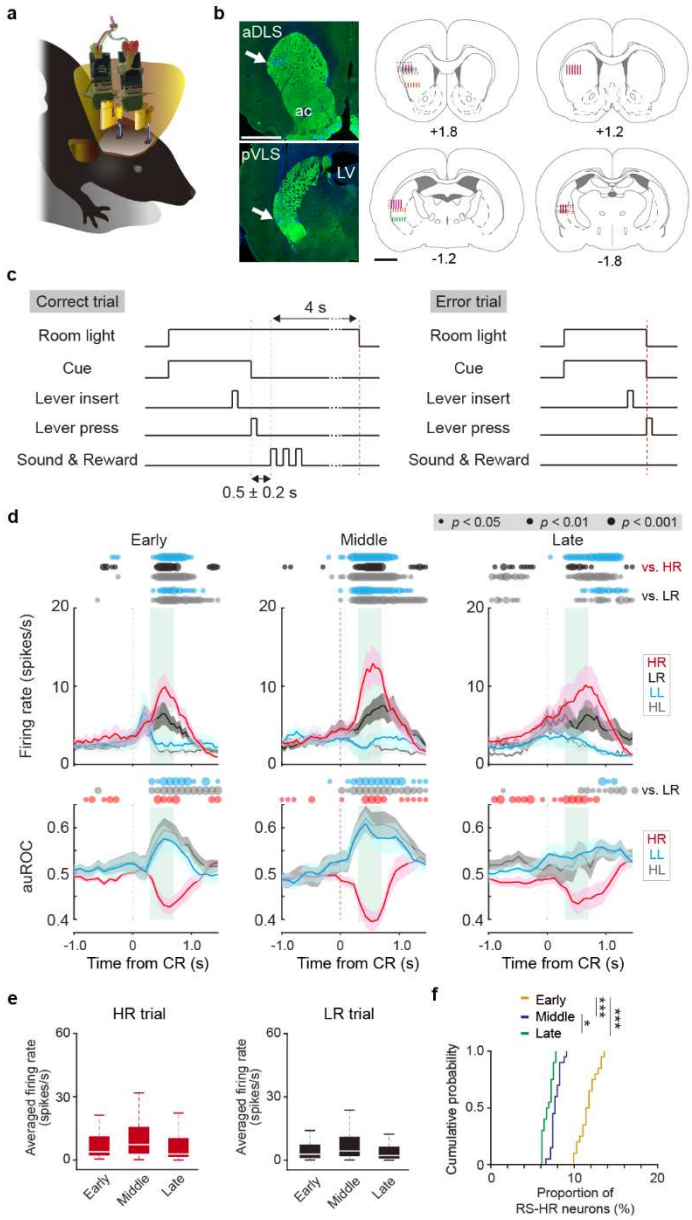
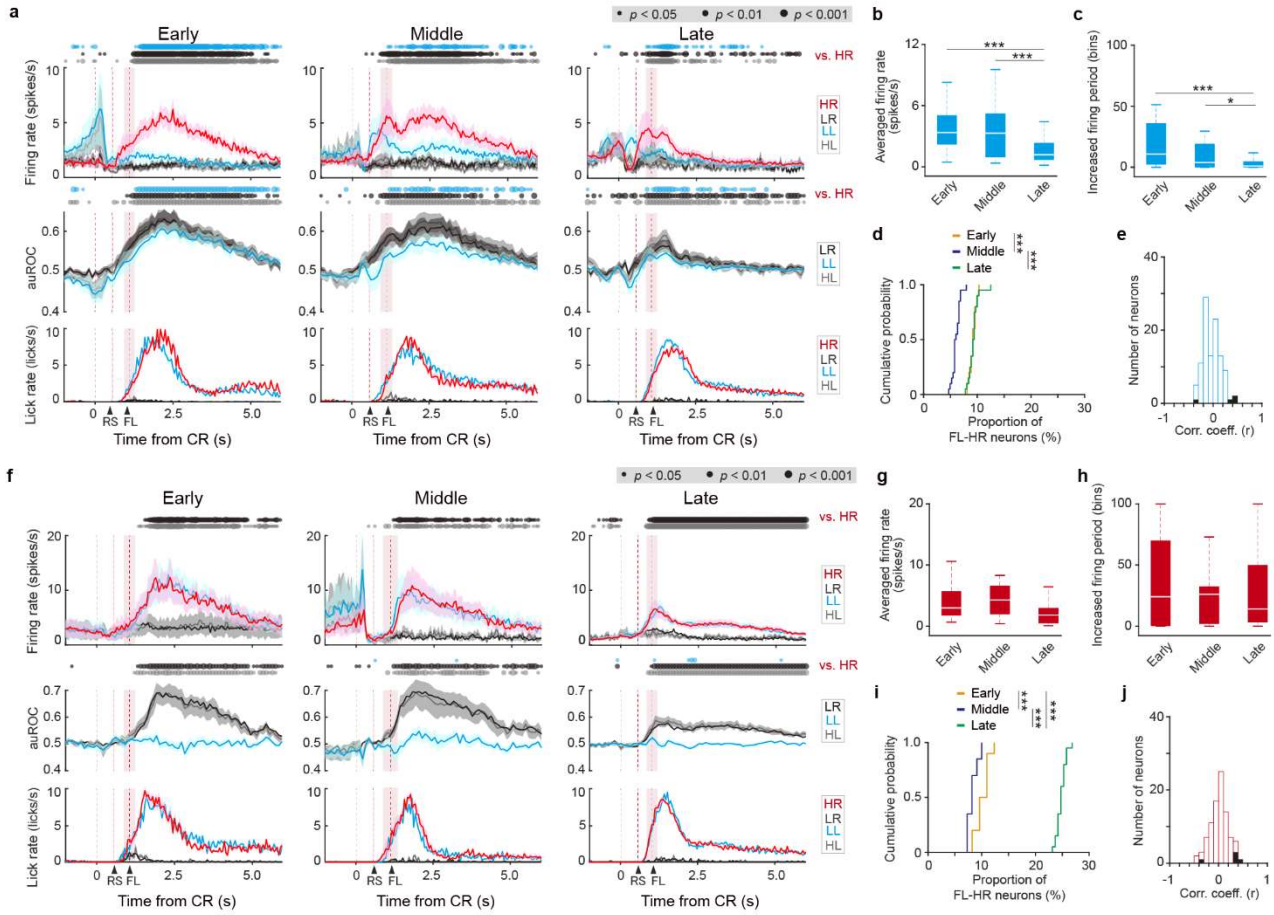


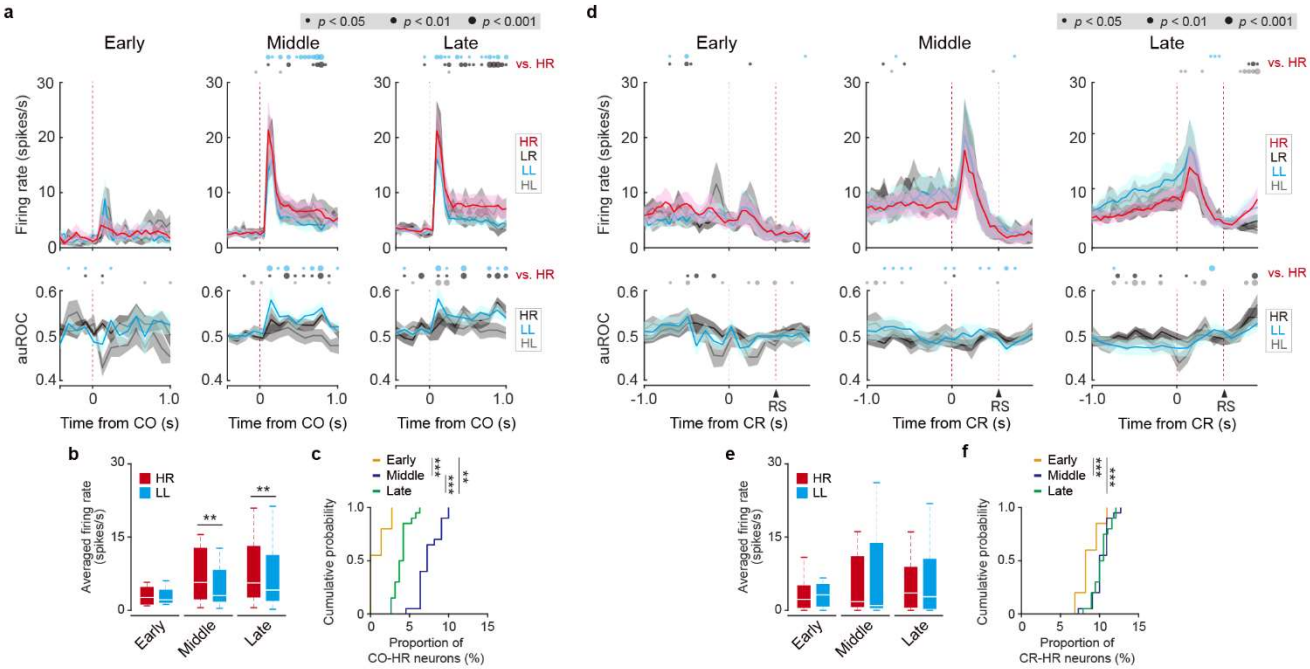
Figure 5. Multi-unit recording of neurons in striatal subregions and firing activity related to the behavioral outcome of RS-HR neurons in the aDLS. a, Schematic illustration of a freely moving rat used for simultaneous multi-unit recordings in the aDLS and pVLS. Six rats were used for the following analysis. **b**, Representative images showing positions of electrode tips (arrows) in the aDLS and pVLS (left rows). The recording sites estimated by electrode tracks and electrical marks in individual rats are shown (center and right rows). ac, anterior commissure; LV, lateral ventricle. **c**, Sequence of some events in correct and error trials. The delay periods were pseudorandomly added between a correct lever press and the reward sound (0.5 ± 0.2 s). The room light turned off the extended time (4 s) after the correct response or immediately after the error response. **d**, Mean firing rate (top rows) and auROC values (bottom rows) of RS-HR neurons in the aDLS during the period when the RS is presented (green shadows, 500 ± 200 ms after the lever press) at the early (n = 43 neurons), middle (n = 37 neurons), and late (n = 44 neurons)

stages. Time bins with significant differences between the mean firing rate in the HR or LR trial and any of the rates in other three trials (Wilcoxon signed rank test) or the distribution of auROC values and 0.5 (Wilcoxon rank-sum test) are represented by the circles at the top. **e**, Averaged firing rate during the RS period in the HR and LR trials at the early, middle, and late stages (Kruskal-Wallis test: HR trial, $\chi^2 = 3.926$, $p = 0.140$; and LR trial, $\chi^2 = 2.812$, $p = 0.245$). **f**, Cumulative probability of the proportion of RS-HR neuron number at the three stages (Kruskal-Wallis test, $\chi^2 = 45.378$, $p = 1.4 \times 10^{-10}$; *post hoc* Tukey-Kramer test, $p = 7.1 \times 10^{-5}$ for early vs. middle, $p = 1.0 \times 10^{-9}$ for early vs. late, and $p = 0.040$ for middle vs. late). Data are indicated as the mean ± s.e.m. (**d**) or the median and quartiles with the maximal and minimal values (**e**). The anteroposterior coordinates from bregma (mm) are shown (**b**). Scale bars; 2 mm (**b**). *$p < 0.05$ and ***$p < 0.001$.



900 **Figure 6. Sustained activity after the reward of FL-HR neurons in the aDLS and pVLS**. **a**, Firing rate (top
901 rows), auROC (middle rows), and licking rate (bottom rows) after the reward of FL-HR neurons in the aDLS at the
902 early (n = 27 neurons), middle (n = 31 neurons), and late (n = 49 neurons) stages. Time bins with significant
903 differences between the mean firing rate in the HR trial and any of the rate in other three trials (Wilcoxon signed
904 rank test) or the distribution of auROC values and 0.5 (Wilcoxon rank-sum test) are represented by the circles at the
905 top. Timings of the RS and FL are shown. **b**, Averaged firing rate for 5 s after the FL in the HR trial (Kruskal-Wallis
906 test, $\chi^2 = 21.748$, $p = 1.9 \times 10^{-5}$, *post hoc* Tukey-Kramer test, early vs. middle, $p = 0.979$, early vs. late, $p = 2.8 \times$
907 10^{-4}, middle vs. late, $p = 4.8 \times 10^{-4}$). **c**, Averaged total number of time bins above 3 S.D. of the baseline firing of
908 aDLS neurons for 5 s after the FL in the HR trial (Kruskal-Wallis test, $\chi^2 = 15.571$, $p = 4.2 \times 10^{-4}$, *post hoc* Tukey-
909 Kramer test, early vs. middle, $p = 0.538$, early vs. late, $p = 6.1 \times 10^{-4}$, middle vs. late, $p = 0.027$). **d**, Cumulative
910 probability of the proportion of FL-HR neuron number (Kruskal-Wallis test, $\chi^2 = 39.280$, $p = 3.0 \times 10^{-9}$, *post hoc*
911 Tukey-Kramer test, early vs. middle, $p = 1.3 \times 10^{-7}$, early vs. late, $p = 0.995$, middle vs. late, $p = 2.3 \times 10^{-7}$). **e**,
912 Distribution of the correlation coefficient between the numbers of spikes of FL-HR neurons and licking. Closed
913 column indicates the number of neurons showing significant correlations between the two parameters (4 out of 107
914 neurons). **f**, Firing rate (top rows), auROC (middle rows), and licking rate (bottom rows) after the reward of FL-HR

915 neurons in the pVLS at the early (n = 17 neurons), middle (n = 11 neurons), and late (n = 64 neurons) stages. Time
916 bins with significant differences between the rate in the HR trial and any of the rate in other trials (Wilcoxon signed
917 rank test) or the distribution of auROC values and 0.5 (Wilcoxon rank-sum test) are represented by the circles at the
918 top. The timings of the RS and FL are shown. **g**, Averaged firing rate of pVLS neurons after the FL in the HR trial
919 (Kruskal-Wallis test, $\chi^2 = 6.810$, $p = 0.033$; *post hoc* Tukey-Kramer test, early vs. middle, $p = 0.988$, early vs. late,
920 $p = 0.064$, and middle vs. late, $p = 0.194$). **h**, Averaged total number of time bins above 3 S.D. of the baseline firing
921 after the FL in the HR trial (Kruskal-Wallis test, $\chi^2 = 0.515$, $p = 0.773$). **i**, Cumulative probability of the proportion
922 of FL-HR neuron number (Kruskal-Wallis test, $\chi^2 = 47.922$, $p = 3.9 \times 10^{-11}$; *post hoc* Tukey-Kramer test, early vs.
923 middle, $p = 0.011$, early vs. late, $p = 1.7 \times 10^{-4}$, and middle vs. late, $p = 9.7 \times 10^{-10}$). **j**, Distribution of the correlation
924 coefficient between the number of spikes in the pVLS and the licking. Closed column indicates the number of
925 neurons showing significant correlations between the two parameters (5 out of 93 neurons). Data are indicated as
926 the mean ± s.e.m. (**a**,**f**) or the median and quartiles with the maximal and minimal values (**b**,**c**,**g**,**h**). *$p < 0.05$ and
927 ***$p < 0.001$.
928



929 **Figure 7. Transient activity related to the beginning and ending of a behavior of CO-HR and CR-HR neurons**
930 **in the pVLS. a**, Firing rate (top rows) and auROC values (bottom rows) of CO-HR neurons in the pVLS at the early
931 (n = 4 neurons), middle (n = 21 neurons), and late (n = 26 neurons) stages. Time bins with significant differences
932 between the mean firing rate in the HR trial and either of the rate in other three trials (Wilcoxon signed rank test) or
933 the distribution of auROC values and 0.5 (Wilcoxon rank-sum test) are represented by the circles at the top. **b**,
934 Averaged firing rate of CO-HR neurons in HR and LL trials (0-600 ms after the CO) (Wilcoxon signed rank test;
935 early, p = 0.875, middle, p = 0.006, and late, p = 0.005). **c**, Cumulative probability of the proportion of CO-HR
936 neuron number (Kruskal-Wallis test, χ^2 = 51.114, $p = 8.0 \times 10^{-12}$; *post hoc* Tukey-Kramer test, early vs. middle, p =
937 9.6×10^{-10}, early vs. late, p = 0.002, and middle vs. late, $p = 6.4 \times 10^{-4}$). **d**, Firing rate (top rows) and auROC values
938 (bottom rows) of CR-HR neurons in the pVLS at the early (n = 11 neurons), middle (n = 22 neurons), and late (n =
939 41 neurons) stages. Time bins with significant differences between the mean rate in the HR trial and any of the rate
940 in other trials (Wilcoxon signed rank test) or the distribution of auROC values and 0.5 (Wilcoxon rank-sum test)
941 are represented by the circles at the top. The timing of the RS is presented. **e**, Averaged firing rate of CR-HR neurons
942 in HR and LL trials (0-600 ms after the CR) (Wilcoxon signed rank test; early, p = 0.424, middle, p = 0.614, and
943 late, p = 0.259). **f**, Cumulative probability of the proportion of CR-HR neuron number (Kruskal-Wallis test, χ^2 =
944 13.786, p = 0.001; *post hoc* Tukey-Kramer test, early vs. middle, p = 0.003, early vs. late, p = 0.005, and middle vs.
945 late, p = 0.976). Data are indicated as the mean ± s.e.m. (**a**,**d**) or the median and quartiles with the maximal and
946 minimal values (**b**,**e**). **p < 0.01 and ***p < 0.001.