Video Article

New Variations for Strategy Set-shifting in the Rat

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Abstract

Behavioral flexibility is crucial for survival in changing environments. Broadly defined, behavioral flexibility requires a shift of behavioral strategy based on a change in governing rules. We describe a strategy set-shifting task that requires an attentional shift from one stimulus dimension to another. The paradigm is often used for testing cognitive flexibility in primates. However, the rodent version has not been as extensively developed. We have recently extended an established set-shifting task in the rat[1] by requiring attention to different stimuli according to context. All the experimental conditions required animals to choose either a left or right lever. Initially, all animals had to choose on the basis of the location of the lever. Subsequently, a change in the rule occurred, which required a shift in set from location-based rule to a rule in which the correct lever was indicated by a light cue. We compared performance on three different versions of the task, in which the light stimulus was either novel, previously relevant, or previously irrelevant. We found that specific neurochemical lesions selectively impaired the ability to make particular types of set shift as measured by the performance on the different versions of the task.

Video Link

The video component of this article can be found at https://www.jove.com/video/55005/

Introduction

Behavioral flexibility is a key requirement for survival in a changing world. One of the established behavioral paradigms for testing this ability is set-shifting, in which a shift of attention from one stimulus dimension to another is necessary for changing action strategies after a change in rule. Several brain regions such as the prefrontal cortex and striatum are implicated in set-shifting[2,3,4,5]. Neural mechanisms for this function have been investigated across several species including humans[5], monkeys[6] and rats[1,7,8,9]. However, the rat versions of set-shifting tasks have not been as extensively developed. The cost-effectiveness of rats, their appropriate size for stereotaxic surgery, and the availability of recently developed genetic methods[10], motivate further development of set-shifting paradigms for use in rats.

A typical set-shifting paradigm for rats requires a change between two behavioral strategies: for example, a response strategy and a visual-cue strategy. Rats initially have to choose one of two available options (such as left or right levers in an operant automated version[1] or left or right arms in a T-maze version[7,8,9,11]). After a set shift, they have to switch to using a visual-cue strategy, such as a light cue indicating the correct side. In those conventional set-shifting tasks, it is necessary to shift attention from one stimulus dimension to another dimension that had been previously irrelevant.

In addition to changing to a dimension that had been previously irrelevant, there is also the logical possibility that a stimulus was previously relevant, or previously absent and now novel. Real life situations in nature may entail attention to a novel, or historically relevant but not crucial cue. Therefore, we considered these subtypes of set-shift, in a new variation of rodent set-shifting based on a previously established automated set-shifting task[1].

We have recently demonstrated the use of the new version of set-shifting paradigms in an experiment to determine the effect of neurochemically specific lesions of the striatum[12]. In our previous study, we targeted cholinergic interneurons releasing acetylcholine (ACh) of the dorsomedial or ventral striatum since ACh and those subregions have been implicated in behavioral flexibility. All the experimental conditions demanded the same strategic shift but each involved different types of attentional shift: to a novel, previously relevant or previously irrelevant cue. We here describe detailed procedures of the paradigms, and highlight representative results suggesting that striatal cholinergic systems play a fundamental role in set-shifting, which is dissociable between different striatal subregions depending on behavioral contexts[12].

Protocol

All procedures for the use of animals were approved by the Animal Care and Use Committee at the Okinawa Institute of Science and Technology.

1. Animals

1. Obtain male Long-Evans rats (250-300 g on arrival).
2. Upon arrival, house a group of two or three rats together for a week and later separate them into individual cages. Note that this experimental design involves food-restriction and needs to hold an animal in each cage to control the amount food consumed.
3. Provide all animals with food and water *ad libitum*, and house them under standard conditions (12 hr/12 hr light/dark cycle, at 23 °C).
4. 5 days before the beginning of behavioral experiments, food-restrict animals at approximately 85% of their average weight with free access to water throughout experiments.
5. Handle animals for 5 min a day for a minimum of 5 days before the beginning of experiments in order to familiarize them with an experimenter.

2. Hardware and Software for Behavioral Tests and Analyses

1. **Hardware**
    1. Use an operant chamber equipped with a sound attenuating box.
    2. The chamber is incorporated with several additional attachments: two receptacle levers on the front panel, two light cues just above the levers, a magazine spot with a sensor for detection of head entry between two levers, a food dispenser, a pure tone generator and a house light on the rear panel.

2. **Software**
    1. Control all the behavioral events under a programmed code written by Trans IV software. Use Med-PC IV software to signal and detect all the behavioral events during training and a test.
    2. Use Trans IV for writing code for behavioral testing. Open a new file for writing.
    3. Once the program is written, translate and compile it within the software. Modify the code if errors are detected and retry.
    4. Upon successful debugging of a program, check if user's code works properly by conducting a trial-run before actual initiation of experiments.
    5. Using MED-PC IV, run behavioral experiments. Open the software, click "open session" and assign a single program to each box.
    6. After all the programs have been properly assigned to each box, send signals necessary to start experiments.
    7. After the completion of the task, save data as clicking "Save Data" or write code to automatically save data (for details, see programmer's manual for MED-PC IV).
    8. Once data is exported to a designated folder, rename the data as acquisition date and animal ID numbers for later use. The collected data is imported into Matlab for behavioral analyses described below (Protocol, 3.7).

3. Behavioral Training and Testing

1. **Habituation and Magazine training.**
    1. Present no levers to animals during those phases.
    2. During the habituation phase, place animals in an operant chamber for 20 minutes a day. On the same day, give 10 sucrose pellets (45 mg) to animals in their home cage, which familiarize them to the sucrose reward.
    3. Next, begin magazine training. Place animals in the chamber and give 20 sucrose pellets (1 pellet per a minute) to the animals, providing an opportunity to learn the location of the food tray and acquisition of the pellets.
    NOTE: Those phases can be skipped due to potential confusion of animals on obtaining a pellet without an operant response, even though we adopted them to make animals familiarized with a chamber and eating from a food tray.

2. **Continuous reinforcement schedule**
    1. Train animals on a continuous reinforcement schedule to obtain a reward by pressing a lever. A session of this training lasts until 60 pellets have been received (60 lever-presses) or 40 min has elapsed.
    2. Present either the left or right lever throughout the first half of a session (until 30 pellets are obtained), followed by the presentation of the opposite lever during the second half. The order is alternated on a daily basis.
    3. Continue this reinforcement schedule until animals have successfully obtained 60 rewards for at least 2 consecutive days. Note that there may be high variability among animals of how quickly they make the first lever-press and this may affect the progress of reinforcement. In this case, put a few sucrose pellets on a presented lever to motivate them to approach toward the lever when no response has been made over the first session.
    NOTE: A possible alternative is to put sucrose pellets when the lever is first presented to animals in order to make the completion of this phase faster.

3. **Time schedule for a trial in lever-press training and testing.**
    1. Start a single trial with a 3 sec (s) tone.
    2. 2 sec after the termination of tone, present two levers and allow animals to press either lever within 10 sec. In case that no response is made within 10 s, retract both levers and count this trial as an omission trial.
    3. In the case of the lever press training, present only a single lever on either side.
    4. In most of experimental conditions, there was a light stimulus above one of levers. Turn the light cue on immediately after the tone ceases and turn off when animals have either made a response or in 10 sec after lever insertion when no response has been made.
    5. Set inter-trial intervals at 20~30 sec.

4. **Lever-press training**
    1. In this training phase, present no light to animals.

2. Train animals under lever-press training for 5-8 sessions with the same time schedule for a trial as testing sessions described below.
3. In this training, present either left or right lever randomly, and the lever has to be pressed within 10 sec after the lever is presented, or the trial is counted as an omission without a response. A session for the lever press training comprised of 80 trials.
4. Once the animals have scored fewer than 10% of omissions out of 80 trials, move them to the following side-bias test.

5. **Side-bias test**
   1. Perform a side-bias test to determine the animal's preference to either left or right lever[1]. A trial entails two lever-presses on both sides.
   2. Place animals in an operant chamber and allow them to choose either lever. In the next attempt, animals have to select the opposite lever in order to obtain a reward. If animal's second attempt is on the same side of the first response, give no reward and continue the trial until a response is made on the opposite side.
   3. Conduct a total of 7 trials to determine animal's side preference.

6. **Testing.**
   1. A daily session consists of 80 trials.
   2. Prepare three different conditions for set-shifting procedures as shown in **Figure 1**.
   3. All the three conditions require animals to similarly change behavioral strategies from choosing one lever that is consistently on the same side (Phase 1, response strategy) to following a light cue that indicates a correct side (Phase 2, visual cue strategy).
   4. Begin with initial learning of a response strategy (Phase 1) for 4 sessions, in which animals have to press a lever based on the location of levers. In this phase, set the correct side to the lever opposite to their preference based on a preliminary side-bias test as mentioned above.
   5. Next, commence visual cue learning (Phase 2) for 10 sessions. A light cue illuminated above either lever indicates a correct lever. In this shifting phase, three different patterns of attentional shifts can be compared among three experimental conditions as described below (3.3.6-3.3.8).
   6. In set-shift condition 1 (**Figure 1A**), give no light in phase 1, but a light cue indicates the correct side in phase 2. In condition 1, therefore, animals need to attend to a novel stimulus.
   7. In set-shift condition 2 (**Figure 1B**), present a light cue above the correct lever in phase 1, and again in phase 2. In this condition, the light cue had been relevant, but not necessarily required for making a choice in phase 1. Thus animals have to attend to a previously relevant cue.
   8. In set-shift condition 3 (**Figure 1C**), turn on a light cue randomly above either left or right lever in phase 1. Thus it has to be ignored. In phase 2 animals are required to pay attention to the light stimulus that has been previously irrelevant.

7. **Behavioral analysis.**
   1. Throughout sessions, measure the percentage of correct responses on a daily basis, excluding omission trials.
   2. Count errors accumulated during 10 sessions of visual cue learning and classify them into perseverative, regressive or never-reinforced errors as described in a previous study[1]. There, a detailed analysis of error types suggests separate functions in set-shifting.
   3. Define perseverative errors as incorrect responses to the previously correct lever while animal's performance was still below chance level[4,6,13,14,15]. Similar criteria have been used in previous studies[1,3,7,11].
   4. Based on a principled way, determine the criterion to separate between perseverative and regressive errors as the point at which the animal first scored fewer than 8 out of 10 incorrect responses (probability of making 8/10 errors or more = 0.054, based on cumulative binomial distribution) in a moving window of 10 trials.
   5. In order to find this point, start calculating a moving average of the 10 trial window from 1st trial and then advance it by one trial at a time until <8/10 errors are measured. Conduct this analysis across all trials in which a light cue is illuminated above the previously incorrect lever during visual cue strategy.
   6. Define all the subsequent errors committed after this point as regressive errors.
   7. During visual cue learning, count never-reinforced errors when animals responded to a previously incorrect lever on which the light cue was not illuminated. Divide them into an early or late portion based on the learning phase; errors made in the first half of 10 sessions (session 1-5) are considered early and those in the second half (session 6-10) are regarded as late ones.

Representative Results

We used the strategy set-shifting task described above to investigate the role of cholinergic interneurons in behavioral flexibility. We compared the effect on the task of an immunotoxin-induced selective lesion of cholinergic interneurons in dorsomedial (DMS), ventral striatum (VS) and saline-injected control. All animals had to switch from choosing a lever based on the side (left or right), to choosing based on a cue light above the correct lever. We used three experimental conditions of set-shift in which the cue light was either: (1) novel, (2) previously relevant (indicating the correct lever), or (3) previously irrelevant (randomly assigned).

In these three experimental conditions, the initial acquisition of response strategy was intact in all the treatment groups, suggesting that cholinergic loss in the striatum had no effects on initial learning (**Figure 2A**-**2C**). These results are consistent with previous studies showing that inactivation of DMS or VS did not affect initial discrimination[7,9] and that application of cholinergic antagonists systemically[16] or locally to the striatum[17,18,19] left initial learning intact.

In set-shift condition 1 (**Figure 2A**, novel cue), the percentage of correct responses was not significantly different. However, the number of perseverative errors was significantly increased in the VS lesion group than controls. During set-shift condition 2 (**Figure 2B**, previously relevant cue) neither learning performance nor types of errors were altered by the lesions. In contrast, in set-shift condition 3 (**Figure 2C**, previously irrelevant cue), the number of perseverative errors was significantly different between groups. In particular, there was a significant increase in perseverative errors after DMS lesions. Compared to the control group, the number of never-reinforced errors was significantly decreased in both DMS and VS lesion groups, which was evident in the early but not in the late phase of visual cue learning.

In summary, VS cholinergic lesions disrupted a strategic shift when a novel stimulus was given as a new important cue, causing more perseverative errors. On the other hand, DMS lesions affected set-shifting only when attention to a previously irrelevant stimulus was required, resulting in a different distribution of error types.

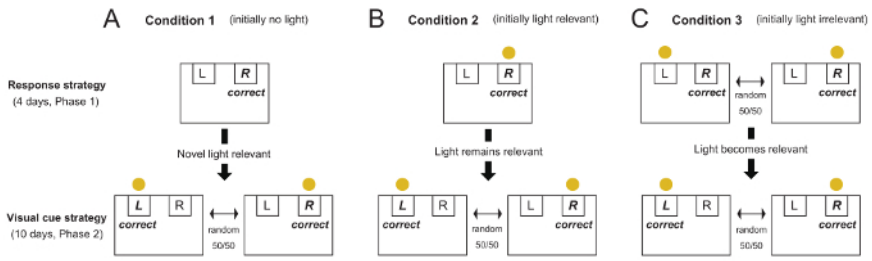

Figure 1: Three different conditions for a set-shift. A flow chart of three variations (**A**, **B** and **C**) of the set-shifting paradigm. A yellow circle shows a visual cue. Reprinted with permission from Aoki *et al.*[12]. Please click here to view a larger version of this figure.

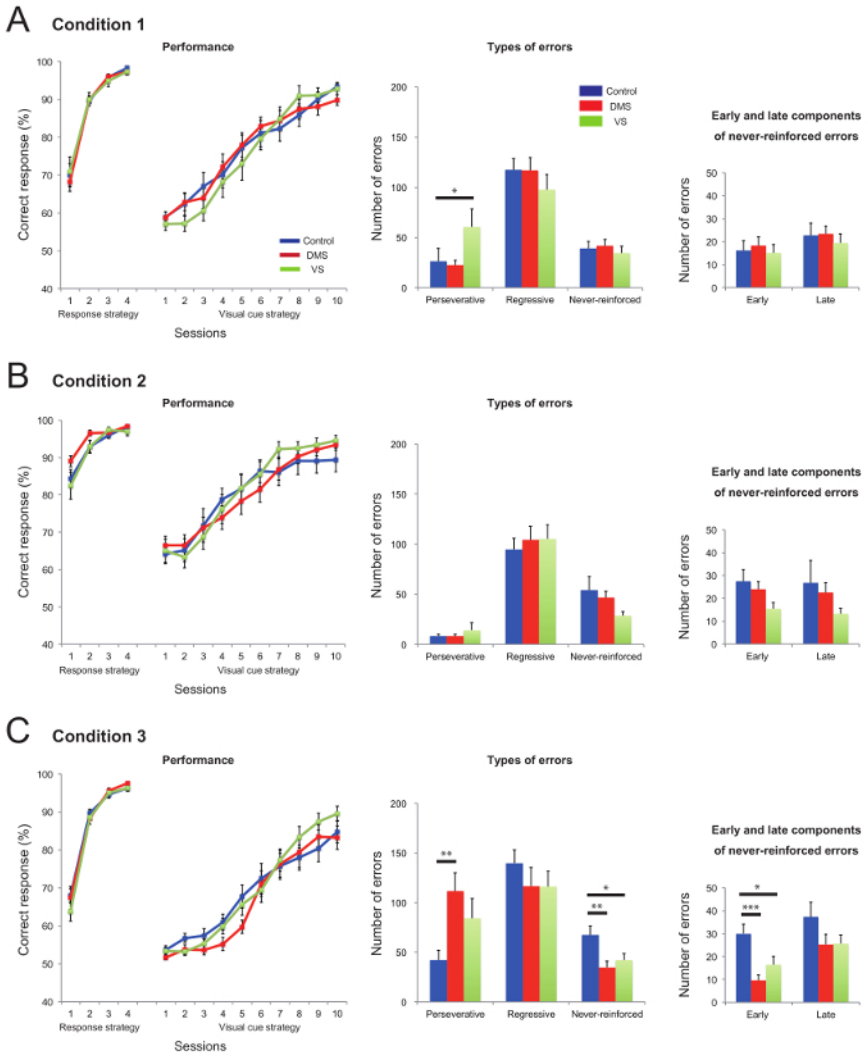

Figure 2: Behavioral results in the set-shifting task. Percentage of correct responses in both response and visual cue strategy (left), types of errors committed over 10 sessions of visual cue strategy (middle), and early and late components of never-reinforced errors (right) are shown for each experimental condition (**A**, a shift of behavioral strategy required attention to a novel stimulus, **B**, to a previously relevant stimulus, **C,** to a previously irrelevant stimulus). Final group size is follows: condition 1, n = 16 (control), n = 19 (DMS), n = 14 (VS); condition 2, n = 13 (control), n = 18 (DMS), n = 14 (VS); condition 3, n = 21 (control), n = 17 (DMS), n = 16 (VS). Data are shown as Means ± SEM. Asterisks *, **, and *** are p <0.05, <0.01 and <0.001, respectively. Reprinted with permission from Aoki *et al.*[12]. Please click here to view a larger version of this figure.

Discussion

We developed new variations on the established set-shifting paradigm for use in rats. Using those paradigms, cholinergic lesions of the striatum were found to impair set-shifting, suggesting a specific role of striatal cholinergic interneurons in set-shifting: suppression of an old rule and facilitation of exploration for a new rule. The effects differed between dorsomedial and ventral striatum, in accordance with the different role of these structures in learning.

A set-shifting task has been widely used to test behavioral flexibility in species ranging from humans to rodents[1,4,5,7,8,9,12]. The term, "set" is defined as the property of the stimulus relevant to behavior in a given trial[20,21]. The present study introduced new variations in which a subject was required to change behavioral strategy based on a change in which set is relevant. The new versions should be compared carefully with other studies using set-shifting. In a typical set-shifting paradigm, a subject initially forms a relevant set to guide behavior and ignores the irrelevant set. After the set-shift, the subject has to attend to the previously irrelevant set. Among the three conditions we proposed here, only condition 3 involves a set-shift. Condition 1 and 2 differ from such set-shift tasks in that either a novel stimulus or a subset of a compound stimulus becomes relevant. Learning curves and the number of perseverative errors of intact rats revealed differences in the initial acquisition and reacquisition between three conditions. Thus, each condition measures different functions: acquisition of a response to a novel cue, attention to a relevant but not crucial cue, and attention to the irrelevant cue. These new variations are useful for investigating neural mechanisms for different forms of behavioral flexibility.

Rats have many advantages for studying the neural mechanisms underlying behavioral flexibility, including their large size making them suitable for stereotaxic surgery, the availability of transgenic strains, and cognitive ability. Previous studies have established T-maze based or automated version of the set-shifting task in rats[1,7,8,9,11]. In case that an automated version is not available, three different manipulations introduced in this article are applicable to a T-maze based set-shift task[3,7]. Also, other stimulus dimensions with different sensory modality such as an odor cue can be combined[22], which further extends variations.

It has previously been shown that inactivation of DMS or VS impairs set-shifting when it requires attending to a previously irrelevant stimulus[7,9]. This is also the case in condition 3 of the present study. However, an important question which remains to be answered is whether impaired set-shifting is derived from being unable to alter action strategies (such as from a response strategy to a visual cue strategy) during a shift, or the inability to pay attention to a stimulus that had been irrelevant in the initial discrimination. It is impossible to decide between these two possibilities by examining a single experimental context only. To dissociate the specific attentional deficit from more general impairment of shifting strategies, we have sought to create new variations of the set-shifting task, using two different conditions requiring the same shift but different types of attention.

Using these additional conditions, we could separate neural substrates underlying a shift of strategies in different contexts. For example, perseveration of VS lesioned rats in condition 1 where a new stimulus was introduced allowed us to reveal a potential mechanism of ventral cholinergic system in attentional processes and approach to the novelty important for the new rule. On the other hand, we did not observe general effects of DMS lesions on a strategic shift. Rather, it was specific to the situation in which a stimulus contingency changed and animals needed to pay attention to a previously irrelevant cue. Two additional conditions successfully control for a general impairment of shifting strategies. This enabled us to conclude that DMS and VS cholinergic systems have a common role in suppression of an old strategy and facilitation of exploratory behavior, even though they work in different environmental contexts, and neither one has a general role in shifting strategies itself.

In conclusion, new set-shifting variations make it possible to analyze rat's cognitive flexibility in more detail and help further understanding of neural mechanisms for behavioral flexibility under different environmental contexts. Future studies testing the involvement of other key brain regions such as prefrontal cortex and hippocampus would be encouraged using a variety of contexts as introduced in this article.

Disclosures

The authors have nothing to disclose.

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