12-11-2017

Reversible cooling-induced deactivations to study cortical contributions to obstacle memory in the walking cat

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Video Article

Reversible Cooling-induced Deactivations to Study Cortical Contributions to Obstacle Memory in the Walking Cat

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Abstract

On complex, naturalistic terrain, sensory information about an environmental obstacle can be used to rapidly adjust locomotor movements for avoidance. For example, in the cat, visual information about an impending obstacle can modulate stepping for avoidance. Locomotor adaptation can also occur independent of vision, as sudden tactile inputs to the leg by an expected obstacle can modify the stepping of all four legs for avoidance. Such complex locomotor coordination involves supraspinal structures, such as the parietal cortex. This protocol describes the use of reversible, cooling-induced cortical deactivation to assess parietal cortex contributions to memory-guided obstacle locomotion in the cat. Small cooling loops, known as cryoloops, are specially shaped to deactivate discrete regions of interest to assess their contributions to an overt behavior. Such methods have been used to elucidate the role of parietal area 5 in memory-guided obstacle avoidance in the cat.

Video Link

The video component of this article can be found at https://www.jove.com/video/56196/

Introduction

On naturalistic, uneven terrain, sensory information about an obstacle, which can be acquired via vision or touch, can rapidly modify locomotion for avoidance. This careful coordination of stepping movements involves multiple cortical regions[1,2]. For example, areas of motor cortex[3,4] and parietal cortex[5,6,7] have been implicated during complex locomotor tasks such as obstacle avoidance. In quadrupedal animals, step modulations required for obstacle avoidance must extend to both the forelegs and hindlegs. If forward locomotion is delayed between the foreleg and hindleg obstacle clearance (which may arise as an animal treads carefully through a complex, naturalistic environment stalking prey), information about the obstacle maintained in the memory is used to guide the hindleg stepping over the obstacle once walking resumes.

Experimental techniques aimed to deactivate discrete cortical areas can be used to study cortical contributions to memory-guided obstacle locomotion. Cooling-induced cortical deactivation provides a reversible, reliable, and reproducible method for assessing cortical contributions to an overt behavior[8]. Cryoloops made from stainless steel tubing are shaped specific to the cortical area of interest, ensuring highly selective and discrete deactivation of loci. Once implanted, chilled methanol pumped through the lumen of a cryoloop cools the region of cortex directly beneath the loop to <20 °C. Below this critical temperature, synaptic transmission is inhibited in the region of the cortex directly beneath the loop. Such deactivation can be reversed simply by ceasing the flow of methanol. This method has been used to study cortical contributions to sensory processing and behaviors[9,10,11,12,13,14,15,16,17], as well as the motor control of saccadic eye movements[18] and memory-guided obstacle locomotion[19].

The purpose of this protocol is to use reversible cooling-induced deactivations to assess the involvement of the parietal cortical areas for locomotor coordination in the cat. Specifically, memory-guided obstacle locomotion was examined with or without active parietal cortex. These methods have been used to successfully demonstrate the role of parietal area 5 in memory-guided obstacle avoidance in the walking cat[19].

Protocol

All procedures were conducted in compliance with the National Research Council's Guide for the Care and Use of Laboratory Animals (eighth edition; 2011) and the Canadian Council on Animal Care's Guide to the Care and Use of Experimental Animals (1993), and were approved by the University of Western Ontario Animal Use Subcommittee of the University Council on Animal Care.

The following procedure can be applied to experiments studying cortical contributions to locomotor control in the walking cat.

1. Apparatus

1. Construct the apparatus used to assess obstacle memory.
   NOTE: The apparatus consists of a 2.43 m long, 29 cm wide walkway enclosed by 18 cm high clear acrylic walls (**Figure 1**). A narrow slot halfway along the apparatus allows a 25.8 cm wide x 3 mm thick obstacle to be raised onto or removed from the walkway using a lever mounted underneath the walking surface.
2. To ensure that attention of the animal is maintained on eating, avoid using the hand to raise or lower the obstacle. Instead, the obstacle can be raised or lowered using the experimenter's leg to move the lever underneath the walkway, allowing the experimenter to continue feeding the animal.
3. Properly maintain the lever system to ensure that the obstacle can be raised or lowered soundlessly.
4. Use a small elevated platform (23 cm long x 23 cm wide x 16 cm high) upon which soft food is placed, to guide movements of the animal.
5. Record all trials using an ethernet camera (54 frames/s) mounted on a tripod 1.85 m away from the midline of the walkway.

2. Training Procedures

NOTE: For successful data acquisition, a period of training preceding behavioral testing ensures that each animal is properly acclimated to the testing room and apparatus. Repeated exposure to a novel environment will aid in reducing startling or other stressful behaviors. Acclimation may vary between animals and may require 1-2 months of training. Initial acclimation sessions may be up to 5 min in length depending on the focus and motivation of the animal to eat. Subsequent sessions should aim to increase the duration of time that the animal is motivated to work (typically around 20-25 min).

1. Acquire mature (>6 months of age) domestic short hair cats from a commercial laboratory breeder of any weight or sex.
   NOTE: Motivation to work for food and a cooperative disposition comprise the selection criteria when considering which animals should be included in the study.
2. Acclimate each animal to wearing a harness to which a 1 m long leash is attached. Anchor the leash to a shelf above the walkway over the midpoint of the walkway.
   NOTE: This allows the animal to walk along the central portion of the apparatus without any tension, thus encouraging the animal to remain within this portion of the apparatus. Establishing such boundaries is helpful for working with a moving test subject.
3. Place the animal onto the walkway, allowing it to eat from the platform upon which soft food is placed.
   NOTE: One aim of this initial training is to ensure that the animal readily follows the food platform when moved forwards, and can walk comfortably with the harness and leash. The use of soft food as positive reinforcement encourages the animal to remain focused throughout each training or testing session, and promotes a comfortable working environment.
4. Ensure that the animal is comfortable with handling, including instances where the animal must be moved to the start area of the walkway.

3. Behavioral Training and Testing Protocol

NOTE: The obstacle memory is assessed in two paradigms: a visually-dependent obstacle memory task, and a tactile-dependent obstacle memory task. Both paradigms should be used during initial training and subsequent testing.

1. **Visual obstacle memory**
   1. To assess the visual obstacle memory, raise the obstacle onto the walkway (**Figure 2A**). Place the platform on the far side of the obstacle. Place the animal in the start area of the walkway.
   2. Allow the animal to approach the food, stepping over the obstacle with only its forelegs in order to eat from the platform.
   3. As the animal continues to eat, lower the obstacle such that it becomes flush with the walkway to prevent any further visual or tactile inputs.
   4. Following a variable delay period, move the food forwards again to encourage the animal to resume walking; this delay can be less than 1 s to upwards of 2 min.
   5. Importantly, perform trials where the obstacle is absent in order to prevent habituation to the obstacle and development of a learned avoidance response. In such visual obstacle-absent trials, ensure that the obstacle is not raised onto the walkway before placing the animal in the start area of the walkway.
   6. Observe hindleg stepping in obstacle-present and obstacle-absent trials to verify typical locomotor behaviors and intact visual obstacle memory prior to cooling. Ensure that the animal can clear the obstacle without contact, and that stepping of all four legs is significantly elevated in obstacle-present trials.
      NOTE: Watching videos of training trials may assist in this verification.

2. **Tactile obstacle memory**
   1. To assess the tactile obstacle memory, ensure that the obstacle is not raised onto the walkway before placing the animal in the start area of the walkway (**Figure 2B**).
   2. Allow the animal to walk towards the food platform placed on the far side of the obstacle slot.
   3. As the animal eats, raise the obstacle onto the walkway beneath the food dish, preventing any visual input of the obstacle.
   4. As the food is moved forwards, note that the animal should contact the obstacle with their forelegs before stepping over it.
   5. Allow the animal to continue eating while straddling the obstacle between their fore- and hindlegs. During this time, lower the obstacle so that it becomes flush with the walkway to prevent any further visual or tactile inputs.
   6. Following a variable delay period, move the food forwards once again to encourage the animal to resume walking.
   7. Importantly, perform trials where the obstacle is absent and no foreleg contact occurs for preventing habituation to the obstacle and development of a learned avoidance response.

1. In these tactile obstacle-absent trials, have the animal approach and eat from the food platform, as described in step 3.2.1. However, raise and lower the obstacle (step 3.2.2) before moving the food forward in step 3.2.3. Ensure that a similar delay period where the animal is allowed to continue eating (step 3.2.4) precedes the final continuation of locomotion (step 3.2.5).

8. Observe the hindleg stepping in the obstacle-present and obstacle-absent trials to verify normal locomotor behaviors and intact visual obstacle memory prior to cooling.

4. Video Analyses

NOTE: To assess obstacle memory, analyses during initial training and subsequent testing after cooling loop implantation involve quantifying the peak step height, step clearance, and the horizontal distance between the toe and obstacle at the peak of each step for both visual and tactile paradigms (**Figure 2C**).

1. Analyze the videos using custom written scripts.
2. For every trial, track each foot by marking the position of the toe closest to the camera throughout each step.
3. Measure the peak step height as the perpendicular distance between the toe and the surface of the walkway at the highest point in each step trajectory (**Figure 2C**).
4. In the obstacle-present trials, measure the step clearance as the step height directly above the obstacle slot subtracted by the height of the obstacle.
5. Additionally, measure the horizontal distance between the toe and the obstacle at the peak of each step in the obstacle-present trials.
6. Confirm that the obstacle memory capabilities are intact prior to the cooling loop implantation by verifying that the peak step height is elevated in the obstacle-present trials in comparison to stepping in the obstacle-absent trials.

5. Cooling Loop (Cryoloop) Implantation

1. Implant cryoloops bilaterally over areas 5 and 7 according to previously reported surgical procedures[8] (**Figure 3**).
2. In brief, for each hemisphere, perform a craniotomy and durotomy from Horsley-Clarke coordinates[20] A15 to A25 to expose the juncture of the ansate and lateral sulci.
3. Position individual cooling loops shaped from 23-gauge stainless steel hypodermic tubing with the loop in direct contact with the cortical surface of parietal area 5 or 7.
4. Secure the base of each cryoloop to the skull with dental acrylic anchored to the stainless steel screws.
5. Close the craniotomies with additional dental acrylic; draw up the skin margins up to the acrylic edges and suture together.

6. Cortical Cooling Protocol

1. **Experimental setup**
   NOTE: Before bringing the animal into the testing room, the cooling circuit is prepared and tested. The cooling circuit consists of a methanol reservoir with an intake tube (3.2 mm O.D., 1.6 mm I.D.), a reciprocating piston pump, and dry ice bath connected via polytetrafluoroethylene tubing (1.6 mm O.D., 0.5 mm I.D.; **Figure 4**). Additionally, a digital thermometer is required.
   1. Add 500 cc dry ice to 200 mL of methanol in the ice bath. Fit tubing ends snugly over the inlet and outlet of a dummy cryoloop to complete the cooling circuit.
   2. Attach the thermocouple plug to a digital thermometer for continuous temperature monitoring using a cable composed of two male thermocouple connectors and a thermocouple wire. Ensure that the length of this cable is sufficient to reach the head of the animal when one end is plugged into the thermometer.
   3. Turn on the piston pump using the switch.
      NOTE: Methanol should be drawn up from the reservoir, passed through the pump to the dry ice bath where the flowing methanol in the tubing will be cooled to -75 °C. The chilled methanol will then exit the ice bath and run through the attached cryoloop before returning to the methanol reservoir.
   4. Ensure that the pump setting, length of tubing within the ice bath, and length of tubing from the ice bath to the dummy loops are optimal such that the dummy cryoloop temperature can reach a steady state around -5.0 °C.
      NOTE: Such temperatures achieved during this initial setup are often sufficient for achieving test temperatures of 3.0 ± 1.0 °C when the same system is used to cool an implanted cryoloop. Difficulty in attaining sufficient cooling can be solved by adjusting the speed of the pump, increasing the length of tubing submerged within the ice bath, and/or minimizing the length of tubing from the ice bath to the cryoloop.
   5. If necessary, lengthen a section of tubing by threading the end of the tube through a tube end fitting and flange the end of the tube with a flanging tool. Attach tubing of a desired length with a similarly flanged end using a connector.
   6. Verify that all connections are snug and no leaks are present. Once satisfied with the initial setup, switch the pump off, and remove the dummy cryoloop; the circuit is now prepared for a test animal.

2. **Behavioral testing**
   1. Place the animal on the testing apparatus. Slide the harness over the head and secure the strap snugly around the animal. Attach the leash.
   2. Remove the protective cap of the implanted cryoloop to expose the inlet and outlet tubes. Fit tubing ends snugly over the inlet and outlet tubes of the cryoloop. Connect the thermocouple plug to the digital thermometer.
   3. Begin the testing session with a visual (step 3.1) or tactile (step 3.2) obstacle memory trial. Follow with additional trials of all four types (visual obstacle-present, visual obstacle-absent, tactile obstacle-present, tactile obstacle-absent) in a random fashion.

NOTE: A typical testing session consists of a 'warm' block of trials, where memory-guided obstacle avoidance is observed in the absence of cooling to establish baseline measures.

4. Switch on the piston pump, and wait for the cryoloop to reach a temperature of 3.0 ± 1.0 °C (1-2 min). Then, run a 'cool' block of trials after the piston pump has been switched on. During this block of trials, if needed, assess contributions of the cooled area to memory-guided. Ensure that the temperature of the cryoloop is maintained at 3.0 ± 1.0 °C throughout the entire block.
NOTE: All four trial types should be randomly interspersed throughout the block.

5. Run a final 'rewarm' block of trials after the piston pump has been switched off, and the cryoloop has returned to its original temperature.
NOTE: Baseline stepping behavior is re-established during this block. Again, all four trial types should be randomly interspersed throughout the block.

3. **Clean-up**
1. When the behavioral testing is concluded, remove the tubing from inlet and outlet tubes. Be conscious of residual methanol that may drip from the tubing ends and may irritate the animal.
2. Ensure that the protective cap is replaced. Remove the leash and harness before returning the animal to the colony. Trim the tubing ends (3-4 mm) using a tubing cutter to prevent leaky connections on the next testing day.

7. Verifying the Extent of Cooling

1. At the end of behavioral testing, confirm that the extent of deactivation is restricted to the region of cortex directly beneath each cryoloop using previously reported techniques[8].
NOTE: This can be verified with thermocline mapping[12] or with a thermal imaging camera[13,14,19].

Representative Results

This protocol has been successfully used to examine parietal cortex contributions to obstacle memory in the walking cat[19]. In this study, cryoloops were implanted bilaterally over parietal areas 5 and 7 in three adult (>6 months of age) female cats (**Figure 5A**). Animals were assessed in the tactile obstacle memory paradigm in the absence of cooling (warm, control condition), or when area 5 or 7 was bilaterally deactivated.

The representative results from that study demonstrate that when area 5 was bilaterally cooled, hindleg stepping was significantly attenuated in the obstacle-present trials (**Figure 5D**, blue). In the warm condition, the mean peak step height for leading and trailing hindlegs was 9.5 ±2.2 cm and 8.0 ±2.1 cm, respectively. A one-way multivariate ANOVA revealed that when area 5 was cooled, the peak step height for leading and trailing hindlegs was significantly reduced to 4.3 ±2.2 cm (p <0.0001) and 3.4 ±1.4 cm (p <0.0001), respectively. The peak step height of the forelegs in the obstacle-present trials or of any leg in the obstacle-absent trials was not affected by area 5 deactivation. Similarly, the peak step height for any leg in either obstacle-present or obstacle-absent trials did not differ from the warm condition when area 7 was deactivated.

Furthermore, the hindleg step clearance was similarly affected when area 5 was deactivated. In comparison to both warm and area 7 cooled conditions, step clearance was reduced to 4.7 ±2.2 cm in the leading hindleg step (p <0.0001; **Figure 5G**) and −5.6 ±1.4 cm in the trailing hindleg step (p <0.0001). Additionally, step trajectory of the trailing hindleg was affected by area 5 deactivation, as the peak occurred before the obstacle, unlike the stepping in both warm and area 7 cooled conditions (**Figure 5G**).

Altogether, such changes in peak step height, step clearance, and step trajectory indicated profound obstacle memory deficits when area 5 was deactivated. Importantly, as the area 5 deactivation only altered the characteristics of hindleg stepping in obstacle-present trials and did not impair the ability to make stepping movements, these observed changes in locomotion reflect memory, not motor deficits. Furthermore, thermal imaging performed at the conclusion of behavioral testing confirmed that cooling was restricted to area 5 or 7 when each loop was individually cooled for each hemisphere (**Figure 6**). Thus overall, these results demonstrate the contributions of parietal area 5 to memory-guided obstacle locomotion in the cat.
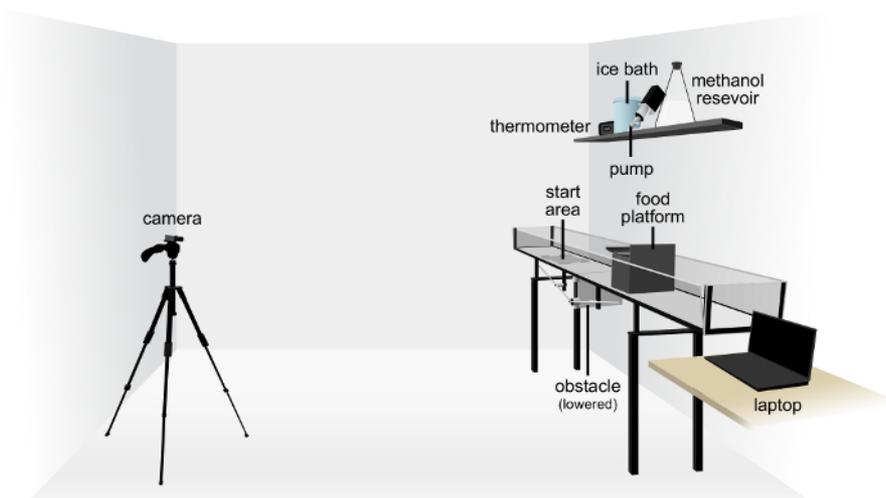
Figure 1: Diagram depicting the camera, cooling equipment, and walking apparatus used to assess obstacle memory in the cat. A 2.43 m long, 29 cm wide walkway is enclosed by 18 cm high clear Plexiglas walls. Halfway along the walkway, a 25.8 cm wide 3 mm thick obstacle can be raised on the walkway through a narrow slot using a lever mounted underneath the walkway. For each trial, the animal is placed a couple of steps from the obstacle in the starting area of the walkway. Food is placed on a small elevated platform (23 cm long x 23 cm wide x 16 cm high) on the far side of the obstacle slot opposite to the starting area. All trials are recorded via an Ethernet camera mounted on top of a tripod and saved on a laptop. This figure has been modified from Wong *et al.*[19] Please click here to view a larger version of this figure.

A Visual Obstacle Memory
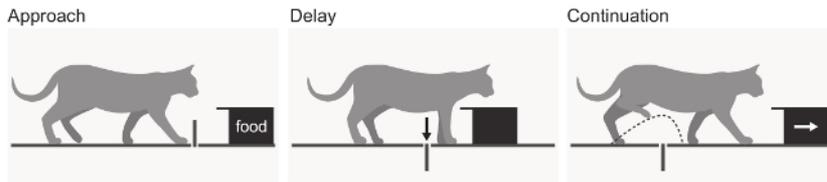
B Tactile Obstacle Memory
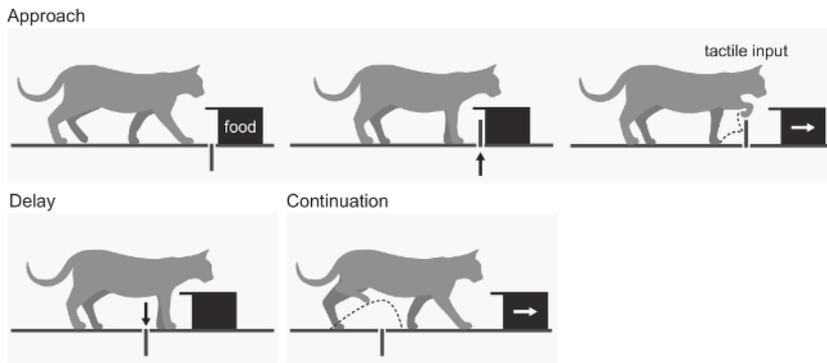
C Step Measurements
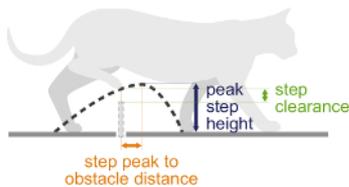
Figure 2: Diagram depicting both visual and tactile obstacle memory tasks and the step measurements used to assess obstacle memory in the walking cat. (**A**) To assess visual obstacle memory, the obstacle is raised onto the walkway as the animal approaches the food platform. After stepping over the obstacle with only its forelegs, the animal is allowed to eat from the platform, as the obstacle is lowered covertly becoming flush with the surface of the walkway. Following a variable delay period, the food is moved forwards to encourage the animal to resume walking. (**B**) To assess tactile obstacle memory, the obstacle is not raised onto the walkway as the animal approaches the food platform. As the animal eats, the obstacle is raised silently onto the walkway directly beneath the food platform. The food is moved forwards causing the forelegs of the animal to contact the obstacle before stepping over it. The animal is allowed to continue eating from the food platform while straddling the obstacle between its forelegs and hindlegs. During this time, the obstacle is covertly lowered from the walkways. The food is moved forward once again to encourage the animal to resume walking. Hindleg steps are measured to assess obstacle memory. (**C**) Stepping is assessed in both visual and tactile obstacle memory paradigms by measuring the peak step height, step clearance, and the horizontal distance between the peak of each step and the obstacle. Please click here to view a larger version of this figure.
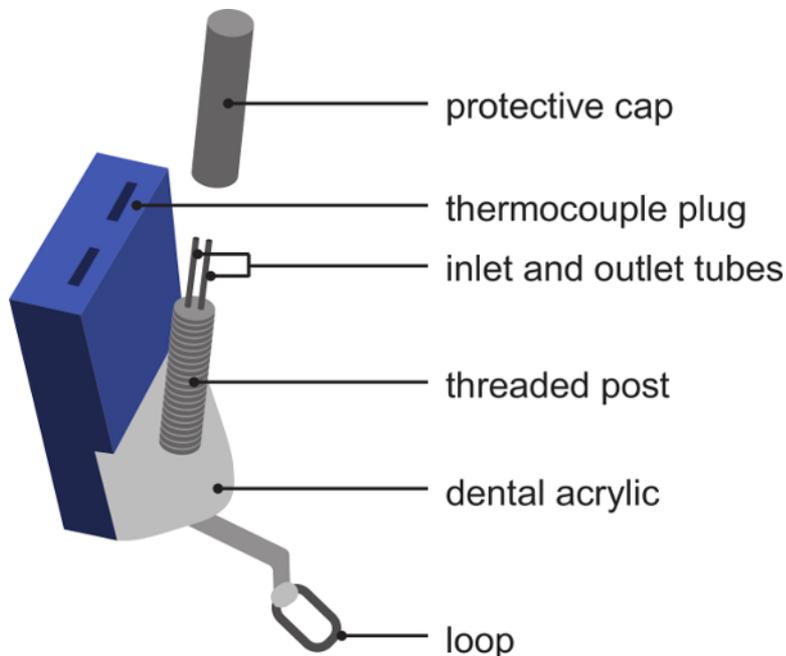
Figure 3: Schematic of the cryoloop. The cryoloop consists of a protective cap, which fits over the inlet and outlet tubes. These tubes run through a threaded post and forms the loop that sits in direct contact with the cortical surface over the region of interest. A microthermocouple is soldered at the union of the loop to measure the cryoloop temperature. Its wires run back up through the heat-shrink tubing (which also wraps the stainless steel tubing) and are attached to a connector. The entire assembly is secured to the skull with dental acrylic. Please click here to view a larger version of this figure.
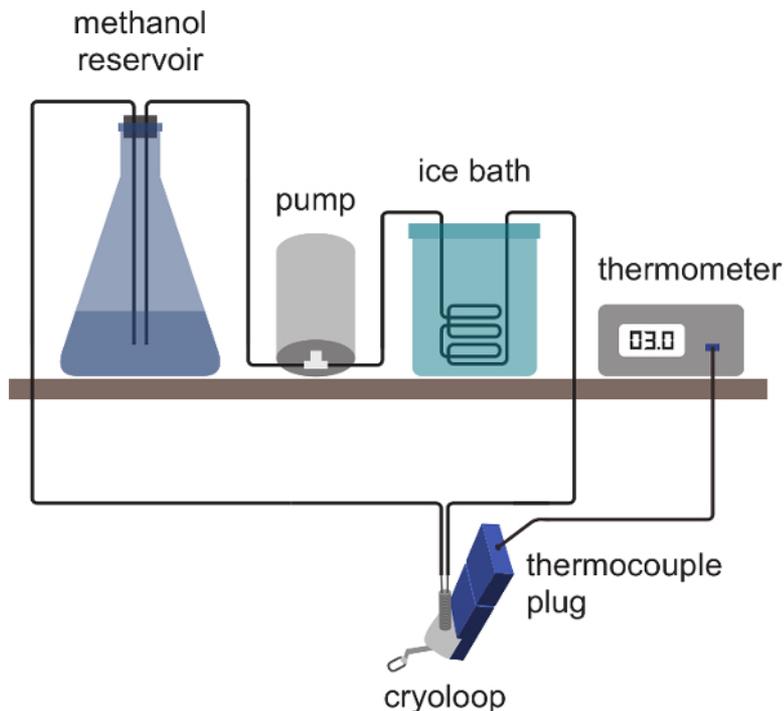
Figure 4: The cooling circuit. The cooling circuit consists of the methanol reservoir, reciprocating piston pump, ice bath, thermometer, and cryoloop. To cool, the pump draws methanol up from the reservoir through the intake tube (1.6 mm I.D.). The methanol exits the pump through the polytetrafluoroethylene tubing (0.5 mm I.D.) and is pumped through to the dry ice bath, where the flowing methanol in the tubing is cooled to -75 °C. The chilled methanol then exits the ice bath and runs through the attached cryoloop before returning to the methanol reservoir. This cryoloop may be a dummy loop (not implanted) used during initial setup, or may be an implanted cryoloop in a test animal. The cryoloop is also connected to a digital thermometer to record loop temperature throughout behavioral testing. Please click here to view a larger version of this figure.
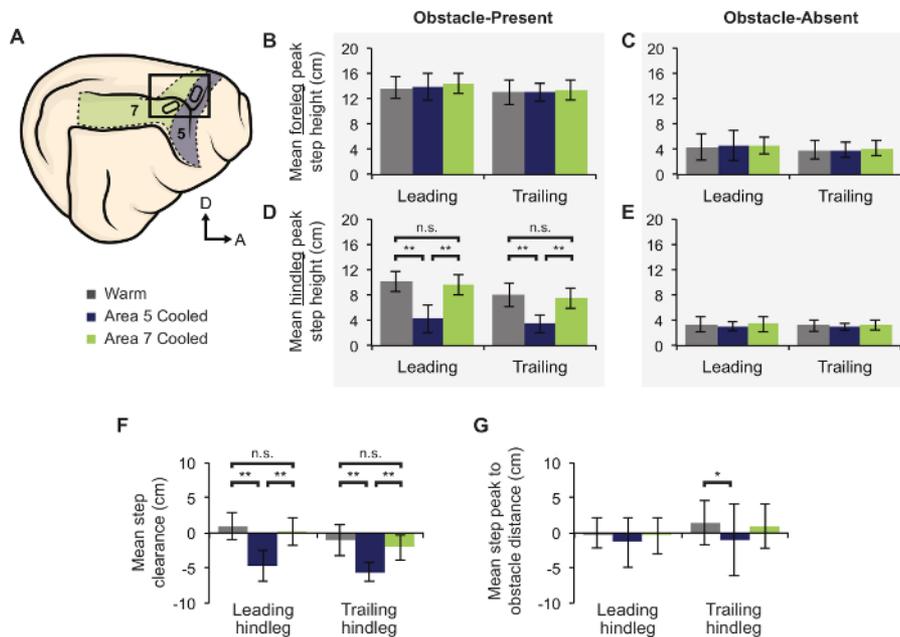
Figure 5: Reversible, cooling-induced deactivation of parietal area 5 results in obstacle memory deficits. (**A**) Lateral view of the right hemisphere of the cat cerebrum showing cryoloops implanted directly over parietal areas 5 (blue) and 7 (green) examined in Wong *et al.*[19] D: dorsal, A: anterior. (**B-E**) Bar plots depicting mean step height ± SD for the obstacle-present (B, D) and obstacle-absent trials (C, E) for the forelegs (B, C) and hindlegs (D, E) for warm (red), area 5 cooled (blue), and area 7 cooled conditions (green). Step height was significantly reduced in both the leading and trailing hindlegs in the obstacle-present trials when area 5 was deactivated. (**F**) Bar plot depicting mean hindleg step clearance ± SD for each cooling condition. Area 5 deactivation resulted in reduced clearance for both leading and trailing hindleg steps. (**G**) Bar plot depicting the mean horizontal distance between the peak of each step and the obstacle for each cooling condition. When area 5 was cooled, step trajectories were more variable and differed significantly from warm and area 7 cooled conditions. *$p < 0.005$, **$p < 0.0001$, n.s.: not significant. This figure has been modified from Wong *et al.*[19] Please click here to view a larger version of this figure.
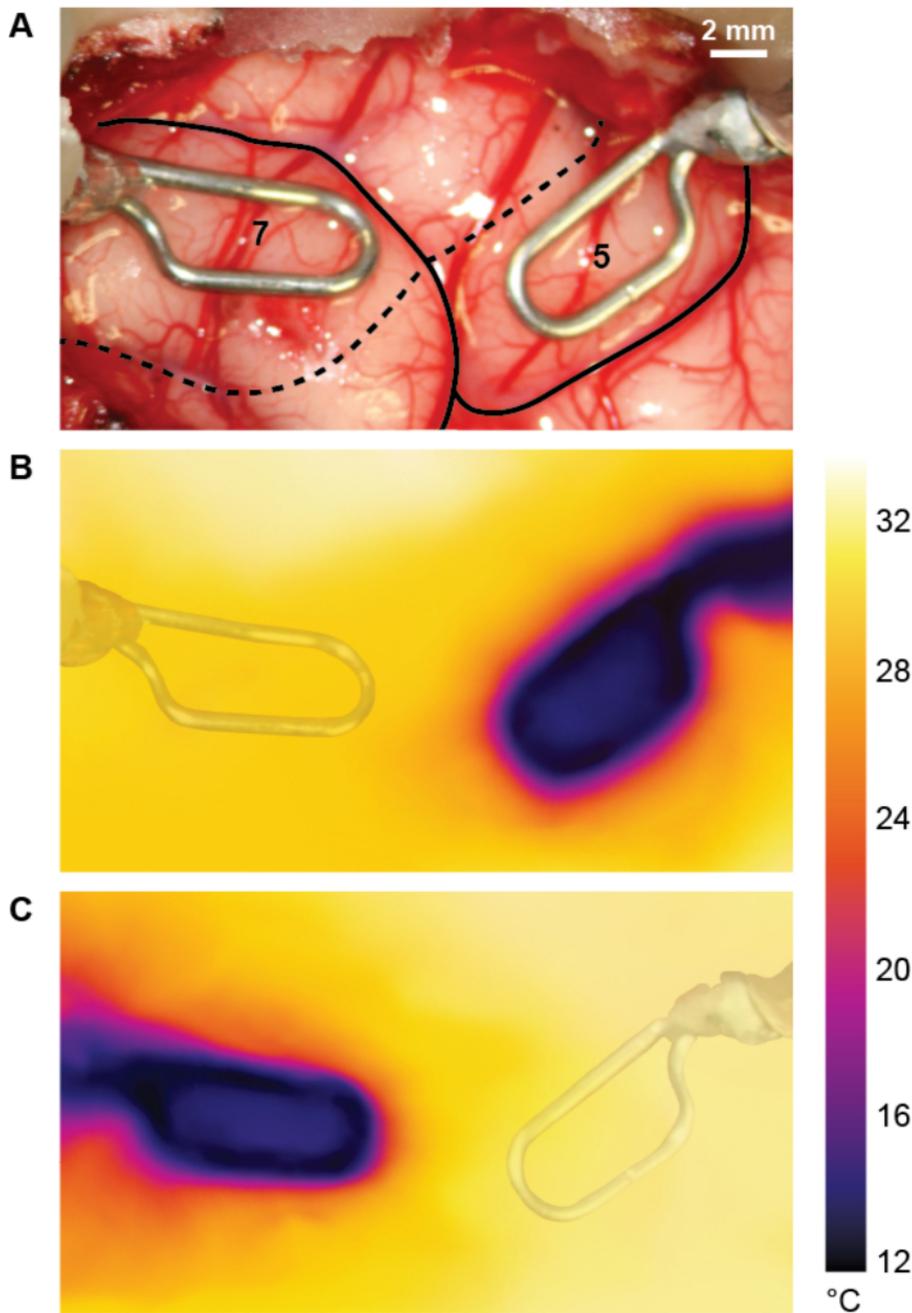
Figure 6: Thermal imaging used to confirm restricted deactivation of area 5 or 7 during cooling. (A) Photograph depicting cryoloops in contact with parietal areas 5 and 7 of the right hemisphere. Top is dorsal, right is anterior. Dashed line represents border between parietal areas 5 and 7. **(B-C)** Thermal images of the parietal cortical surface photographed when the cryoloop over area 5 (B) or area 7 (C) was cooled to 3 °C. This figure has been modified from Wong et al.[19] Please click here to view a larger version of this figure.

Discussion

The described paradigm employs cooling-induced deactivations of discrete cortical areas using the cryoloop in order to study memory-guided obstacle locomotion in the cat. The visual and tactile obstacle memory paradigms are fairly simple for animals to execute as they exploit naturalistic locomotor behaviors that occur with minimal effort when an animal is motivated to follow a moving food source. Thus, the majority of the training period is devoted to acclimating the animal the testing room and cooling equipment. Most animals require repeated exposure to wearing the harness and being tethered via the leash before walking comfortably and naturally on the apparatus. Additionally, during testing, the sound of the piston pump may distract or startle the animal. Completing the cooling circuit with the dummy cryoloop and running the pump during initial training can allow the animal to acclimate to the sound of the pump. Despite sufficient training prior to testing, there will likely be a

limited time for testing before the animal becomes restless. Therefore, ample time is devoted to ensure proper setup and troubleshooting prior to bringing the animal into the testing room will optimize subsequent data collection.

Difficulty attaining sufficient cooling can be addressed by adjusting the pump speed. However, attention should be paid to the increasing pressure that may result with the tubing being forced off the inlet or outlet tubes of the cryoloop. Alternatively, the length of tubing submerged in the ice bath may be increased to enable more time to chill the flow of methanol within the tubes. Additionally, ensuring that the length of tubing from the point of exit from the ice bath to the cryoloop is as short as possible will minimize loss of cooling. However, this distance must also be long enough to allow sufficient range of locomotion for a given behavioral paradigm. Tubing may be insulated with flexible foam wrapping to optimize the cooling efficiency. Such wrapping can also prevent drops of condensation that form around the tubing from falling on the animal, which may irritate or startle the animal. During testing, ensuring a snug fit of the tubing over the inlet and outlet tubes of the cryoloop can make connecting the cryoloop difficult. Wearing a nitrile or latex glove can provide a better grip of the tubing. Ensuring that the animal is comfortable and patient while the experimenter attaches the tubing is essential. Food may be used to keep the animal stationary and content.

Cryoloops can be routinely cooled yielding highly reproducible changes in behavior when a particular area is deactivated. By assessing the same task in the presence and absence of cortical deactivation within the same animal, the overall number of animals used may be reduced. Furthermore, the extent of cooling may be manipulated to further specify cortical contributions to a specific behavior. For example, both unilateral and bilateral deactivations can be performed in the same animal to examine possible lateralization effects of a behavior. Additionally, the degree of cooling can be varied to examine laminar contributions. By cooling cryoloops at the cortical surface to 3.0 ±1.0 °C, all six layers of cortex directly beneath each loop are cooled to <20 °C, inhibiting neuronal spiking activity[22]. Alternatively, cryoloops can be cooled to 8.0 ±1.0 °C, which selectively cools only the supragranular cortical layers below this critical temperature of 20 °C. Assessing behaviors with such superficial cortical deactivation as well as full cortical deactivation may permit translaminar dissociations of cortical function[21].

Despite such versatility, the following limitations should be considered during experimental design. While cooling is an excellent approach for deactivating all cell types in a given cortical region, it cannot provide a means of deactivation with the cellular specificity that may be achieved with optogenetic deactivation techniques. Furthermore, cooling requires a minimum of 45 s before cryoloop temperatures stabilize at the critical temperature of 3.0 ±1.0 °C for functional deactivation. Thus, considerations for the time span required to achieve functional deactivation should be incorporated in the experimental protocol of choice.

Overall, the cooling system requires minimal maintenance. Tubing and connectors of the cooling circuit should be checked regularly for leaks. The methanol within the reservoir should be replaced weekly to ensure that the methanol is free from particulate matter. Implanted cryoloops also require minimal maintenance. The margins are cleaned periodically with a 3% hydrogen peroxide solution followed by a surgical scrub solution. With proper use and care, implanted cryoloops can be cooled routinely for many years. These cortical cooling procedures can be adapted to other behavioral paradigms[10,11,12] or electrophysiological recording preparations[13,14] in alternative animal models[15,17,18,22].

Disclosures

The authors have nothing to disclose.

Acknowledgements

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