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Original Research

Inactivation of Dorsolateral Prefrontal Cortex During Auditory Working Memory

Bethany Plakke[1], Katryna Skye Kevelson[2], Charles Leyens[3], Lizabeth M. Romanski[2,*]

[1]Department of Psychological Sciences, Kansas State University, Manhattan, KS 66502, USA
[2]Department of Neuroscience, University of Rochester School of Medicine, Rochester, NY 14642, USA
[3]Department of Computer Science, Rochester Institute of Technology, Rochester, NY 14623, USA
*Correspondence: Liz_romanski@urmc.rochester.edu (Lizabeth M. Romanski)

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Abstract

Background: The dorsolateral prefrontal cortex (DLPFC) is a critical node in the working memory (WM) neural circuit, established through neurophysiology, neuropsychology, and neuroimaging studies in humans and nonhuman primates. While most of the neurophysiological evidence for the role of the DLPFC in WM comes from visuospatial WM paradigms, evidence for its role in auditory WM has been suggested by the fact that large lateral prefrontal cortex lesions in nonhuman primates cause auditory discrimination deficits. Moreover, DLPFC neurons demonstrate task-related neuronal responses during auditory WM. In contrast, other studies have proposed that the ventrolateral prefrontal cortex (VLPFC) plays a pivotal role in auditory and audiovisual processing, integration, and mnemonic processing, since VLPFC neurons are responsive to complex acoustic stimuli and are robustly active during auditory WM tasks. Furthermore, inactivation of the VLPFC impairs audiovisual and auditory WM. In these inactivation studies the cortical region that was inactivated by cortical cooling included areas 12/47, 45 and 46 ventral. It is possible that inclusion of area 46 ventral may account for the auditory WM performance deficit previously observed while inactivating VLPFC so further experiments are needed. **Methods**: In the present study we examined whether transient inactivation of the DLPFC, including areas 46v and 46d, and 9, in rhesus macaques would effect auditory WM. The DLPFC was inactivated by cortical cooling while two rhesus macaques performed an auditory working memory task. This was followed by permanent ibotenic acid lesions and assessment of behavioral performance post-lesion. **Results**: Our experiments demonstrated that inactivation of DLPFC by cortical cooling in two macaques did not result in a significant decrease in performance of an auditory WM task. The inactivation resulted in an increase in dropped gaze events during the latter half of the task, in one subject, which could be due to a loss of attention or motivation. The ibotenic acid lesions of the DLPFC did not significantly alter performance on the auditory WM task. **Conclusions**: Our results showed that DLPFC transient inactivation with cortical cooling and ibotenic acid lesions did not significantly alter overall auditory working memory performance, which differs from the impairment seen when the VLPFC is inactivated. Our data suggest that the DLPFC and VLPFC may play different roles in auditory working memory.

Keywords: prefrontal cortex; auditory discrimination; working memory; vocalization; lesion

1. Introduction

The lateral prefrontal cortex is involved in working memory, decision making, sensorimotor processing, attentional selection and cognitive control [1–7]. The diversity of its functions makes this area seem a chameleon, able to flexibly support many cognitive functions depending on the context of task or stimuli [8–11]. Since multiple cytoarchitectonic regions make-up the lateral prefrontal cortex, the varied cognitive functions it supports may be executed through cooperation of discrete subdivisions that each contribute to specific functions. Goldman-Rakic and colleagues [12–14], in their Domain Specificity hypothesis, argued that different anatomical subdivisions of the prefrontal cortex (PFC) participate in working memory, each with content-specific processing and storage mechanisms. In particular, extension of the dorsal "where" and ventral "what" visual streams into the dorsal and ventral prefrontal cortex, provided the substrate for spatial and object processing domains, respectively [12–16]. Single neurons recorded in dorsolateral prefrontal cortex (DLPFC) areas 46 and 8a demonstrated a robust representation of visual space in perceptual and mnemonic tasks [16–20] and lesions of these areas caused mnemonic scotomas of these visuospatial locations [21]. Below the principal sulcus, ventrolateral prefrontal cortical (VLPFC) neurons show less selectivity for spatial location and more selective responses for object features [16,22,23] including faces [24–26].

Most of the neurophysiological investigations of prefrontal working memory mechanisms have relied on visual stimulus processing in nonhuman primates despite the importance of the frontal lobe in speech and language in the human brain. Few neurophysiological studies have examined the role of dorsal or ventral PFC in auditory working memory. Early investigations examined the role of the prefrontal cortex in delayed response and in auditory discrimination using large lesions that included portions of both dorsal and ventrolateral prefrontal cortex in Old World monkeys [27–30]. Several studies noted an impairment in visual delayed response performance when lesions included the principal sulcus of the DLFPC. In comparison, lesions which included the inferior convexity, below the principal sulcus, interfered with auditory discrimination [29,31].

Neurophysiological recording studies of DLPFC and VLPFC have demonstrated task-related [32,33] activity during auditory discrimination, auditory working memory or categorization [32,34–38]. Recordings specifically from VLPFC indicate that neurons exhibit selective responses to complex sounds, including vocalizations and some VLPFC auditory neurons respond to complex sounds with similar acoustic morphology [37,39–43], suggesting a topography for acoustic feature processing. During working memory tasks, VLPFC neurons exhibited both task and stimulus-related responses using auditory or audiovisual memoranda [33,35,44]. In contrast, neurophysiological recordings from single neurons in DLPFC demonstrate task-related firing during auditory working memory during several epochs in the working memory task [38]. In this study, few neurons in DLPFC exhibited stimulus-specific responses.

Thus, both DLPFC and VLPFC neurons are activated during neurophysiological recordings during auditory working memory tasks but may play different roles. We have previously used cortical cooling to transiently inactivate VLPFC and found that it impaired working memory during auditory and audiovisual processing [45]. The area which was inactivated and led to WM disruption included areas 12/47, 45 but also ventral 46 (46v). Area 46v is on the border between dorsal and ventral prefrontal cortex. It receives some projections from the auditory parabelt cortex. Thus, the effects of VLPFC inactivation could be due to inclusion of DLPFC area 46v rather than VLPFC alone. Unfortunately, transient inactivation of DLPFC during auditory working memory has not been done so a comparison of the role of these two prefrontal regions in auditory working memory is needed.

To address this issue, we obtained two rhesus macaques from the Plakke *et al*., 2013 study [38] and inactivated the same lateral prefrontal cortical region that had been shown to be active during the auditory feature-based working memory task with neurophysiological recording. The chambers in these two subjects were placed on lateral prefrontal cortex, and included portions of areas 9, 46d, and 46v and are referred to as DLPFC. First, we inactivated DLPFC with transient cortical cooling over multiple behavioral sessions, in the subjects while they performed an auditory working memory task. Secondly, we placed permanent ibotenic acid lesions into the cortex that had been previously recorded in these same rhesus macaque monkeys [38] and evaluated their performance post-lesion. Our results showed that DLPFC transient inactivation with cortical cooling and ibotenic acid lesions did not significantly alter overall auditory working memory performance, which differs from the impairment seen when VLPFC is inactivated. Our data suggests that DLPFC and VLPFC may play different roles in auditory working memory.

2. Materials and Methods

The research subjects were two macaques (1 male, 1 female, Macaca Mulatta), both 19 yrs old at the start of the experiment, previously trained on auditory delayed match-to-sample task (DMTS) at the University of Iowa where bilateral prefrontal recording chambers and a head-post were surgically implanted. The recording chambers included portions of areas 8, 9 and 46 [46,47] and were centered just above the principal sulcus. Single unit recordings in the dorsolateral prefrontal cortex were previously made in both subjects during an auditory DMTS task. This data was presented in [38]. After transfer to the University of Rochester the bilateral cortical chambers were maintained for the present inactivation experiments. All procedures were in accordance with the United States Department of Agriculture (USDA) regulations and were approved by the University of Rochester Committee on Animal Resources.

2.1 Training Procedure

Training sessions and inactivation sessions were performed in a sound-attenuated room lined with acoustic foam (Sonex, Acoustical solutions, Henrico, VA, USA). Visual stimuli were presented to the subjects at eye level, on a computer monitor (NEC MultiSync LCD1830, 1280 × 1024, 60 Hz) which was 75 cm from the subject's eyes. Auditory stimuli were presented via two speakers (Yamaha, MSP5, Shizuoka, Japan; frequency response, 50 Hz to 40 kHz), placed on either side of the computer monitor at the height of the subject's head. The auditory stimuli ranged from 65 to 80 dB sound pressure level measured at the level of the monkey's ear with a B and K sound level meter. Eye position was continuously monitored using an infrared pupil monitoring system (ISCAN ETL200, ISCAN Inc., Woburn, MA, USA). The subjects indicated a correct response by pressing a button, located centrally on the front panel of the chair. Eye position and button press data were collected on a PC via PCI interface boards (NI PCI-6220 and NI PCI-6509; National Instruments, Austin, TX, USA). The timing of stimulus presentation and reward delivery was controlled with in-house C++ software, which was based on Microsoft DirectX Technologies.

2.2 Auditory Task Stimuli

The auditory DMTS task used short audio clips of animals and humans vocalizing as well as nonvocal sounds (hammering, ringing, synthetic noise). The categories of stimuli used in the auditory version of the task included monkey vocalizations, human vocalizations, other animal sounds, and inanimate object sounds. The macaque auditory and visual vocalization stimuli are part of a library of movies and separate audio recordings of monkeys

Table 1. Stimulus lists.

List 1	List 2	List 3
CH_SCREAM	AR_COO	AF_BARK
SM_COO	AR_GRUNT	BV_COO
PH_GRUNT	BQ_GIRNEY	DE_GRUNT
ST_BARK	CH_SCREAM2	DH_HARMONIC
HUMAN "AH"	HUMAN "WE KNOW"	HUMAN "SO"
HUMAN "SHH"	HUMAN "PLACE"	HUMAN "CHANGE"
HUMAN "OOO"	HUMAN "AREA"	HUMAN "TIME"
HUMAN "BEST"	HUMAN "GOES ON"	HUMAN "GUY"
FINGERS SNAP	CHORD	BOTTLE WHISTLE
"BOUNCE"	BIRD CALL	DIGITAL "BLOOP"
FM SWEEP	GLASS "CLANG"	MACHINE "BUZZ"
HORN	"BUBBLES" EFFECT	SODA TOP

The 3 stimulus lists which were used during the performance of the auditory DMTS task are shown. Each list has 4 species-specific rhesus-macaque calls, 4 human vocalizations and 4 nonvocal sounds. The 4 macaque calls in each list are named with an abbreviation for the animal's identity followed by the type of call (i.e., CH_SCREAM, SM_COO, PH_GRUNT, ST_BARK, etc.). DMTS, delayed match-to-sample task.

filmed in our home colony in previous years [33,45,48,49]. The two subjects from this report were not familiar with the animals in the videos. Human vocalization stimuli included unfamiliar male or female actors performing vowel sounds or saying single or a string of monosyllabic words (Oo, Area, How are you). The auditory clips were processed and edited using GoldWave (v5.01, GoldWave Inc., St. Johns, Newfoundland, Canada), Praat (ver. 5.3, free open-source software by Paul Boersma and David Weenink, University of Amsterdam, Amsterdam, The Netherlands) and Audacity (version 3.1, free open-source, https://www.audacityteam.org) software. The movies and their associated audio were extracted using Windows Movie Maker (for Windows 7, Microsoft, Redmond, WA, USA), and Corel Video Studio (ver. Pro X2, Corel Corp., Ottawa, ON, Canada). The duration of the auditory stimuli ranged from 500–1200 msec.

The auditory lists used in this experiment are shown in Table 1. Each list contained four macaque vocalizations (1 coo, 1 scream, 1 grunt, 1 noisy aggressive call); 4 human single spoken words (2 male voices, 2 female voices) and 4 non-vocal sounds. The sample and nonmatch stimuli were from different categories to make the discrimination easier.

2.3 Auditory Delayed-Match-to-Sample Task

To begin a trial, subjects were required to fixate a central square on the screen for 500 ms. At the end of the fixation period, the subject was presented with a sample stimulus, followed by a fixed delay period. On half of the trials, referred to as trial type 1, the second stimulus presented after the delay was a match stimulus, which required a button press. On the other half of the trials, referred to as trial type 2, the second stimulus was an intervening distractor nonmatch stimulus, followed by a second delay period, which was then followed by the match stimulus (Fig. 1). Both these trial types were randomly interleaved to make the presentation of the match stimulus unpredictable. Subjects were required to respond correctly via a button press to the match stimulus to receive a juice reward, which occurred 500 ms after button press. The nonmatch stimulus was always from a different stimulus category than the match stimulus. The delay period for subject 1 was 1000–1100 ms, and for subject 2800 ms. The delay periods were set at a value for each subject to achieve a performance accuracy of >65% correct on average across a training session.

During training, incorrect trials were on a delayed repeat schedule. If a series of more than 5 incorrect trials occurred a time-out was given. If this occurred more than 3 times the training session was modified or ended. The auditory DMTS task is a more difficult working memory task for monkeys than its visual counterpart and performance in the auditory DMTS was inconsistent. Extra training sessions were given after several months of cooling sessions to remediate any performance issues.

2.4 Cooling Apparatus and Procedure

We bilaterally inactivated the dorsolateral prefrontal cortex (DLPFC) while subjects performed DMTS tasks by cooling the cortical surface of DLPFC to 15–20 °C (59–68 °F; Fahrenheit), with methods as described in our previous study [45] and several other prefrontal cooling experiments [50–52]. The cortical surface was cooled via placing stainless-steel, sealed, cooling pots into each recording chamber, which rested on the dural surface. A temperature probe was affixed to the bottom of the cooling pot to mea-

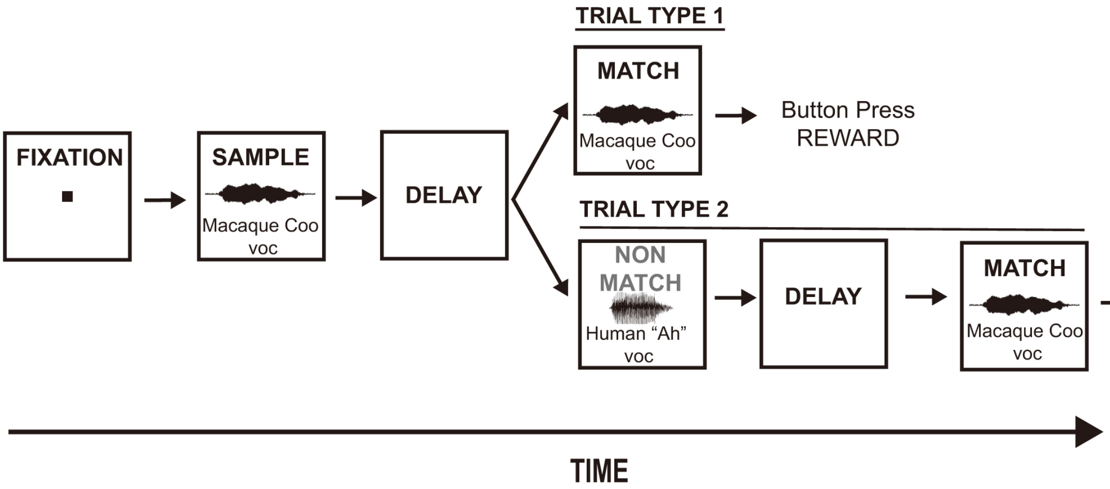

Fig. 1. Auditory delayed-match-to sample task. The timeline for the task is shown. The trial begins when the subject fixates a colored square in the center of the screen. Then the sample auditory stimulus is presented, followed by an 800–1100 msec delay. After the delay, the second or decision stimulus is presented. In type 1 trials the second stimulus is a repetition of the Sample (Match stimulus), and a button press is required to detect this match, then a reward is given and the trial ends. In type 2 trials the second stimulus is a Nonmatch stimulus which differs from the Sample (different category of auditory stimulus). The subject must withhold a button press. Then, a second delay occurs (800–1100 msec) and then the third stimulus will be the Match stimulus (same as the Sample) and will be detected with a button press. Juice reward is given for correct detection of the Match with the button press, and the trial ends.

sure the temperature at the dura. During inactivation sessions, cold ethanol (200 proof Ethyl Alcohol, Part number V1001, Koptec, King of Prussia, PA, USA) (–40 °C to –60 °C) was circulated through the sealed cooling pots to lower the temperature of the cortex through the dura to achieve a cortical target temperature of 15–20 °C (59°–68 °F) to decrease synaptic activity. Temperature of the dural surface and of the circulating ethanol was constantly monitored and recorded throughout the inactivation session. The cooling pots were torus, or donut-shaped, allowing for a central hole (size = 20 gauge) so that a temperature probe could be inserted into the center hole in the cooling pot to confirm the decrease in temperature in the cortex at a depth of 2.5–3.0 mm during cooling. At the end of the cooling session, room temperature ethanol was circulated through the cooling pots to gradually return brain temperature to normal levels (Fig. 2).

2.5 Ibotenic Acid Lesions

After the completion of the cooling studies, we performed permanent bilateral excitotoxic lesions of the prefrontal cortex by placing injections of ibotenic acid (10–15 µg/µL; Cat. No. 0285, Tocris Labs, Bristol, UK) into the cortical area within our recording chambers. The lesion procedures were conducted as a separate aseptic surgical procedure. The subjects were anesthetized using ketamine (0143-9508-10, Ketamine hydrochloride, Hikma Pharmaceuticals, Berkeley Heights, NJ, USA) (3–6 mg/kg) and medetomidine (Domitor, Zoetis, Parsippany, NJ, USA) (0.1–0.15 mg/kg) and placed into a modified stereotaxic frame. The lesion procedures were based on previously published methods of excitotoxic ibotenic acid lesions in rhesus macaques [53,54]. For each injection, 1 µL of ibotenic acid (10–15 µg/µL) was injected into the cortex within the recording chamber using a sterile 20–25-gauge Hamilton syringe. The injections were placed approximately 2 mm apart throughout the 18 mm diameter chambers. In all, approximately 25 injections of ibotenic acid (total of 25 µL) were placed in each recording chamber. After each injection, the chamber was flushed with cool sterile saline to prevent swelling and to remove excess ibotenic acid. After the lesion procedure for one hemisphere was completed, the animal was given a 1–2 weeks recovery period. After the recovery period, the lesion procedure for the other hemisphere was executed, following which the animal was given another 2 weeks recovery before behavioral testing resumed.

2.6 Experimental Procedure

Subjects were first behaviorally trained on the auditory DMTS task to a performance accuracy of at least 68%. After completing behavioral training for 7–13 months, we carried out cooling sessions 1–2 times per week to assess performance during temporary inactivation of DLPFC. During the week, non-cooling days would involve training or control sessions. To begin a cooling session, the recording chambers were cleaned, and lidocaine (USP 5%, 00168-0204, Fougera Pharmaceuticals Inc., Melville, NY, USA) was applied to the dura for 7–10 minutes. After that, any excess lidocaine was cleared and cooling pots

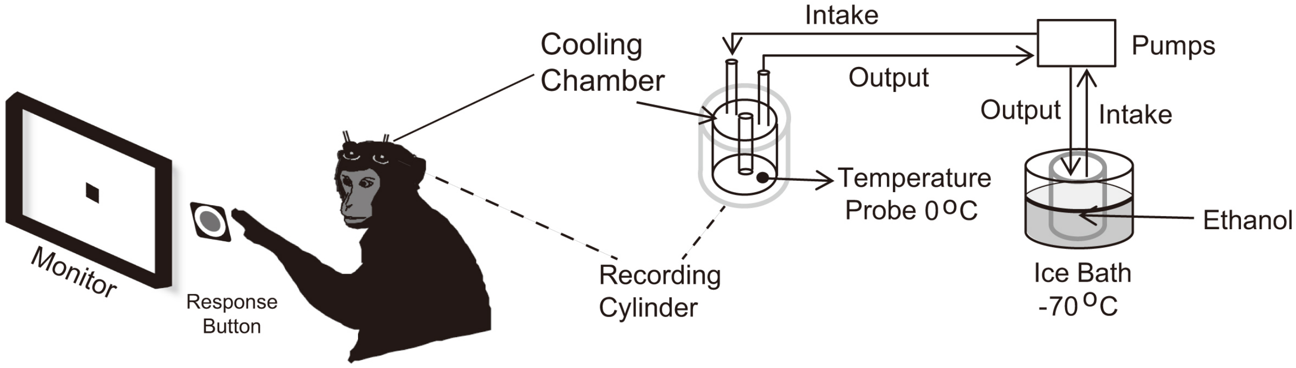

Fig. 2. Cortical cooling method. The subject is seated in front of a monitor with side-flanking speakers. The bilateral DLPFC cortical cylinders are shown on the subject's head. The torus-shaped cooling chambers were placed inside the recording cylinders on the subject's head over the dural surface overlying DLPFC. Cooled ethanol is pumped from a reservoir within a cooling bath and through tubing into the cooling chambers and then returned to the ethanol reservoir. The temperature of the dura is measured with a temperature probe fixed to the bottom of the cooling chamber. Cortical temperature is measured by a different temperature probe inserted through a hole in the center of the cooling chambers into the cortex at a depth of 1–3 mm. Temperature of the circulating cooling solution is constantly monitored and adjusted. DLPFC, dorsolateral prefrontal cortex.

were lowered into the recording chambers, such that the base of the cooling cylinders rested against the dural tissue. The dura was kept free of tissue overgrowth by application once a week with 5-flourouracil (CAS No. 51-21-8, Sigma-Aldrich, Inc., St. Louis, MO, USA) and careful removal of tissue overgrowth with sterile forceps after application of lidocaine.

Within each cooling session, a block of ~100 WARM trials were first carried out to establish baseline performance accuracy. During this block, room temperature/slightly warmed ethanol was circulated through the cooling cylinders. This was followed by a block of 100–150 "cold trials", during which ice-cold ethanol was circulated through the cooling cylinders to inactivate DLPFC. The ethanol was cooled by submerging a beaker of ethanol into a supercooled bath of dry ice (–70 °C) which, when circulated through the cooling pump system, into the sealed cooling pots that were adjacent to the dura, would reduce the cortical temperature to 15–20 °C (59–68 °F). Temperature of the ethanol solution was constantly monitored in the cooling bath, and at the surface of the dura using a temperature probe on the bottom of the cooling pot which lay against the dura. The subject performed the task while the cortical temperature slowly decreased with the cooling process. In post-experiment analysis we designated the "cooling" trials from the time ~7–10 min after the cold solution replaced the warm ethanol and the temperature remained consistently below 10 °C at the level of the dural probes, for several trials. The cooling session ended after the completion of 100 trials or when the subject stopped initiating trials due to satiety. We assessed performance accuracy, and lost fixations/gaze during WARM and COLD trials.

2.7 Control Warm-Warm Sessions

In addition to the cooling sessions, we also conducted sessions to control for possible order effects due to fatigue in the second block, or a practice effect (increase in performance on later trials). For the control sessions, a block of 100 "warm" trials was followed by a second block of 100 "warm" trials. For both the warm blocks, room temperature/slightly warmer, ethanol was circulated through the cooling pots. The number of trials and time interval between the two blocks in the Warm-Warm sessions (time that would be required to cool the brain during a cooling session) was the same as in Warm-Cold cooling sessions. We performed warm-warm control sessions during the same time periods as the cooling experiments on intervening days and then again, as a re-training period prior to ibotenic acid injections.

After completion of the testing sessions for auditory WM during cortical cooling, and follow-up warm-warm sessions, both subjects received permanent bilateral ibotenic acid lesions. After a recovery period of approximately 2 weeks, both the subjects were re-tested in the auditory DMTS task. We carried out 12 post-lesion sessions in subject 1 and 12 post-lesion sessions for subject 2 over a four-week period.

2.8 Data Analysis

Performance was quantified using percent correct for each session. Since the task requires responding only to the match stimulus, and withholding a response to the nonmatch stimulus on trial type 2, the subjects can make the following kinds of errors: "false alarm" error when subjects pressed the button to the nonmatch stimulus on trial type 2, and a "missed press" error when subjects did not press the button to the match stimulus for trial type 1 or did not press to the match stimulus at the end of trial type 2 (Fig. 1). When subjects stopped initiating trials by fixating the starting central point, the session ended. During trials, a large "gaze" window (10–12 degrees) was outlined against the dark screen and subjects were required to maintain their gaze within the window during the trials. If a subject dropped their gaze from the window, the trial was aborted. The number of dropped gaze events was recorded as a measure of attention, motivation or fatigue in the task. The effect of cooling on performance accuracy was assessed with paired *t*-tests and two-way ANOVA models (factors: temperature, trial type) for both animals individually. A similar model was also performed to evaluate effect of experimental manipulations on gaze dropping events. Analyses were conducted for the cooling experiment (block 1 = warm; block 2 = cooling) the control experiment (block 1 = warm; block 2 = warm) and the post-lesion performance assessment (block 1 = warm; block 2 = warm). Statistical tests were carried out using IBM SPSS Statistics (Version 28, IBM Corp., Armonk, NY, USA).

2.9 Lesion Assessment

Upon completion of the post-lesion DMTS testing of both animals, the subjects were euthanized by Euthasol (25 mg/kg, IV; pentobarbital/phenytoin solution, Virbac Corporate, Bridgeton, MO, USA) overdose given after initial sedation with ketamine (10–15 mg/kg, IM). Then a perfusion with 4% paraformaldehyde (Cat.No. S898-07, J.T. Baker Inc., Phillipsburg, NJ, USA) was performed, and the brains were removed, blocked and cryoprotected by submersion in increasing sucrose solutions (10, 20, 30% phosphate buffered sucrose). Frozen sections from the frontal lobes were cut on a sliding microtome at 40 µm. Sections were mounted onto glass slides and stained with thionin (Thionin acetate, Cat.No. A18912.06, Thermo Scientific, Waltham, MA, USA) for Nissl substance. The sections were then examined on an Olympus Provis AX-70 Microscope (Olympus Optical Co. LTD, Tokyo, Japan) to determine the location of the ibotenic acid injections and the boundaries of the cooling chamber location. Evidence of cell loss, degeneration and pyknotic nuclei were seen in the cortex of DLPFC, indicating the location of the ibotenic acid injections and the ensuing neuronal loss. This was charted in NeuroLucida (MicroBrightfield, Inc., Williston, VT, USA) and the sections were aligned with the location of DLPFC areas 8, 9, 46, and 46v in the rhesus monkey atlas [55] and the National Institutes of Health (NIH), "Red" monkey atlas.

3. Results

We performed transient inactivation of DLPFC in two rhesus macaques from the Plakke *et al.*, 2013 [38] study, who were trained in an AWM paradigm. Both macaques had dual recording chambers placed over the DLPFC and had previously had neurophysiological recordings of DLPFC during auditory and visual memory and presentation tasks [38,56]. The version of the task that was used in this study is a DMTS Task [57]. In our version, a sample auditory stimulus is presented, then a delay ensues, then either the matching stimulus is presented (Trial Type 1) or an intervening non-match stimulus is presented followed by a second delay and then the match stimulus occurs just afterwards (Trial Type 2) as depicted in Fig. 1. A button press response is required in both trial types to detect the match, which would be as stimulus 2 in trial type 1 or as stimulus three in trial type 2 (Fig. 1). Juice reward was given for correct responses. Calculation of performance accuracy was done by averaging performance on trial type 1 and 2 for a Total Percent Correct score.

3.1 Testing Protocol

After 7–13 months each animal subject achieved criterion (75% for subject 1 and 67% for subject 2) and the recording chambers were prepared for the cooling experiments. Over a 12-month period, testing sessions were conducted 1–2 times per week and would include 2–3 blocks (100 trials each) of the Auditory DMTS paradigm.

Prior to cooling sessions, the chambers were cleaned and prepared for cooling as described. The sealed cooling pots were placed into the cortical chambers with the temperature probe lying adjacent to the dura in each chamber (Fig. 2). A center hole in the chamber pots allowed for the introduction of a cortical temperature probe which was lowered 3 mm into the cortex on intermittent cooling sessions. The ethanol circulating pumps were turned on when the task began. During the first block which was considered the "Warm" block, the circulating ethanol was at, or slightly above, room temperature and was pumped into the sealed cooling pots as the monkey performed the AWM task. After approximately 100 trials, with the monkey continuing to perform the AWM, cold ethanol was introduced into the pumps and the temperature at the level of the dura, the level of the cortex and the level of the ethanol solution was monitored as it decreased. When the brain temperature reached a stable target level (5–10 °C at the level of the dura and 15–20 °C in the cortex) the beginning of the "Cold" block was noted. Trials during the Cold block were continued until ~100 trials were completed. After ~100 trials the circulating ethanol solution was slowly rewarmed to room temperature or slightly above, and the session was concluded. If the subject stopped initiating trials, or showed any sign of distress the session was stopped, and the circulating solution was rewarmed. In addition to the Warm-Cold Sessions (100 Warm trials and 100 Cold trials) a control session with 100 Warm and a second 100 Warm trial block was performed on alternate days. This was done to control for the effect of fatigue or a practice effect, during the second block of trials, during the cooling sessions.

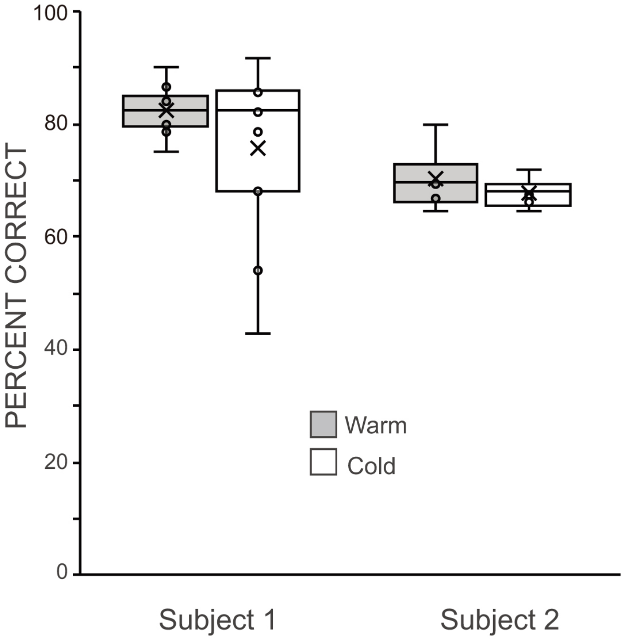

Fig. 3. Overall performance accuracy during inactivation of DLPFC in 2 rhesus macaques during auditory match to sample task. There was no significant difference between the cooling sessions (COLD, white) and the control (Warm, Grey) sessions (paired *t*-test: subject 1, t(11) = 1.626, $p = 0.126$ (two-tailed) and subject 2: t(5) = 1.49, $p = 0.197$ (two tailed). In the bar and whisker plot the mean is denoted with an "x" and the median is portrayed by the horizontal line with the first quartile below and the third quartile above this line. Max and min values are indicated by the vertical lines (whiskers) extending above and below the box.

3.2 Experiment 1—Inactivation of DLPFC With Bilateral Cortical Cooling

A total of 12 Warm-Cold testing sessions passed criterion for analysis for subject 1 and 6 Warm-Cold for subject 2. We used the performance rate in the initial warm session as an index of reliability and removed testing sessions which had a performance score of less than 68% correct for subject 1 and 65% for subject 2 in the beginning Warm session since this early low performance was less than our established criterion. We analyzed the Warm-Cold DLPFC inactivation sessions in subject 1 and in subject 2 using a paired *t*-test on performance for the effect of temperature. In both subjects the effect of inactivating DLPFC by cooling was not significant (Fig. 3).

In subject 1 a paired samples *t*-test was conducted to compare differences in performance by the subject during the baseline testing block at normal temperature (WARM, mean = 82.5% (± 1.2% SEM)) and then during the cooling inactivation (COLD, mean = 76% (± 4.2% SEM)). The results showed a non-significant difference (t(11) = 1.626, $p = 0.132$). The effect size, calculated as Cohens' *d*, using the mean difference divided by the standard deviation of the differences, was = 0.469 [confidence interval (CI): –0.138, 1.058]. In subject 2, the warm block mean was 70% (± 2.2% SEM) and the cold block mean was 68% (± 1.3% SEM). A paired *t*-test showed a non-significant difference (t(5) = 1.486, $p = 0.197$), and the Cohens' *d* was = 0.5 [CI: –0.297, 1.462]. In both subjects, an effect size >0.4 suggests a medium effect size and a potentially noticeable difference [58]. Due to the small sample size, Hedges' g which mitigates the bias of small sample sizes was applied and found to be >0.2 (subject 1, g = 0.43 and subject 2, g = 0.5). Further research with larger sample sizes and increased testing sessions are suggested.

The performance in the DMTS task is a combination of the responses in trial type 1 and trial type 2. In general, subjects tend to make more errors during type 2 trials which require inhibiting a press to the nonmatching stimulus [33]. Thus, we assessed the effect of cortical cooling by trial type with a two-way ANOVA by temperature (warm, cold sessions) and trial type (1 and 2). In both subjects the interaction of temperature and trial type was not significant (subject 1, F(1, 44) = 1.97, $p = 0.168$, $\eta^2 = 0.043$; subject 2, F(1, 20) = 1.138, $p = 0.299$, $\eta^2 = 0.54$). The main effect of temperature was not significant in either subject (subject 1, F(1, 44) = 1.55, $p = 0.220$, $\eta^2 = 0.034$); subject 2, F(1, 20) = 0.163, $p = 0.690$, $\eta^2 = 0.008$). In both subjects, performance was significantly lower in trial type 2 than in trial type 1 (subject 1, F(1, 44) = 58.31, $p < 0.001$, $\eta^2 = 0.570$; subject 2, ($p = 0.007$, F(1, 20) = 8.92; $\eta^2 = 0.308$) (Fig. 4), but this did not vary by temperature as shown by the lack of an interaction effect.

3.3 Experiment 2—Warm-Warm Control Sessions

Since the inactivation with cortical cooling involves 2 blocks of testing over a single session it is possible that any effects seen could be due to a practice effect with better performance in the second session or an effect of fatigue with a worsening of performance and increased variability in the second session when motivation may be lower and could adversely affect performance. To confirm that this did not occur in our experiment we performed a series of control sessions termed Warm-Warm, where the cooling pots were placed into the bilateral chambers over DLPFC and the cooling pumps were turned on, but the temperature of circulating ethanol was kept at, or slightly above, room temperature. The warm-warm sessions were done on alternate days to the cooling sessions. A total of n = 12 warm-warm control sessions were completed for subject 1 and n = 8 sessions for subject 2. In subject 1 the average percent correct in warm block 1 was 85% ± 2.4% SEM and that of warm block 2 was 88% ± 2.5% SEM (Fig. 5). For subject 2, performance in the first warm block was 70% ± 1.5% SEM and in the second warm block the percent correct was 68% ± 1.9% SEM (Fig. 5). A paired *t*-test indicated that there was no significant difference between the two consecutive blocks in subject 1, t(11) = –1.57, $p = 0.145$ (two-tailed) as well as in subject 2, t(7) = 0.80, $p = 0.447$ (two-tailed).

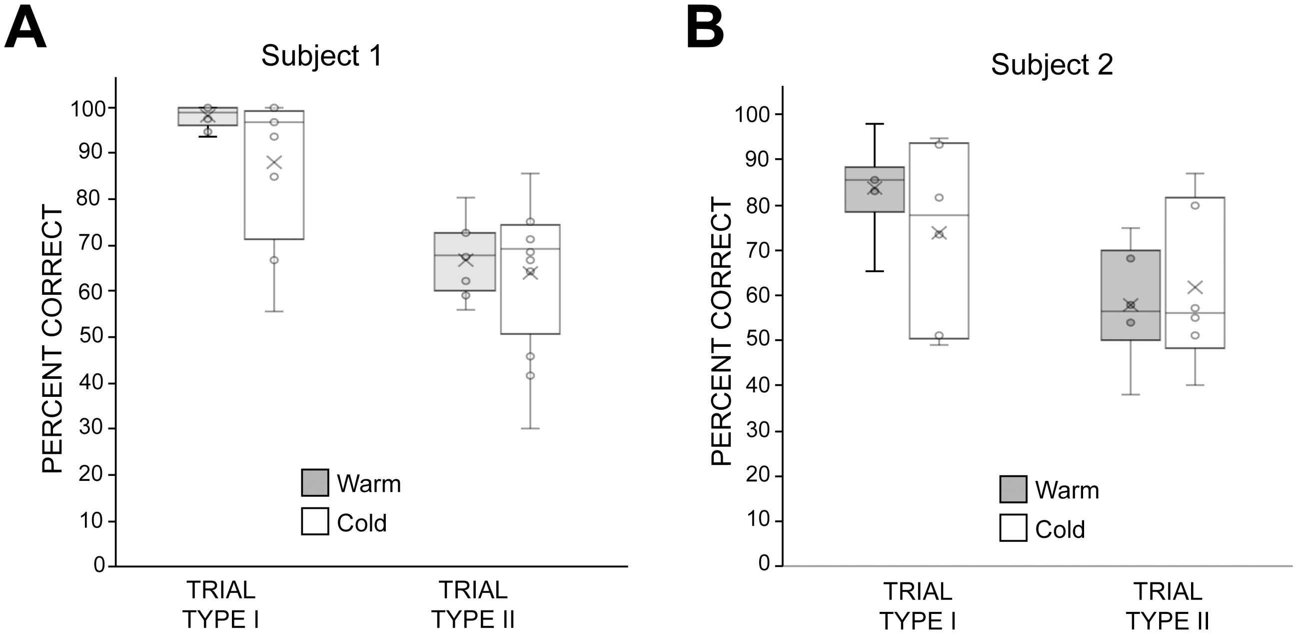

Fig. 4. Performance accuracy across trial types. The performance accuracy of subject 1 (A) and subject 2 (B) during cooling for each trial type is shown with warm trials in gray and the cold trials in white. In both subjects the interaction of temperature and trial type was not significant, nor was the main effect of temperature. There was a significant effect of trial type in both subjects (subject 1, $p < 0.001$; subject 2, $p = 0.007$). In the bar and whisker plot the mean is denoted with an "x" and the median is portrayed by the horizontal line with the first quartile below and the third quartile above this line. The vertical lines are the maximum and minimum (whiskers) extending above and below the box. The observations are indicated by the open circles and the max and min observations are the "whiskers" on the vertical bars.

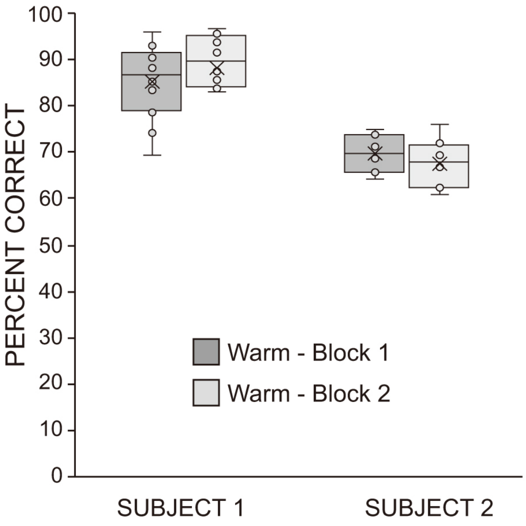

Fig. 5. Control Sessions. Warm—Warm Control Sessions are shown for both subjects where the experimental sessions were identical in all respects to Experiment 1, except that the temperature of the circulating ethanol in the cooling system was kept close to body temperature in both the first and second blocks. There was no significant difference between the early block (block 1, light grey) and the later block (block 2, dark gray) indicating no effect of temporal order on performance accuracy.

3.4 Experiment 3—Ibotenic Acid Lesions

For experiment 3 we used injections of ibotenic acid across the cortical surface within the cooling chamber to examine the effect of a permanent lesion of DLPFC on auditory DMS performance. Two weeks after the ibotenic acid injections in DLPFC were completed, we resumed testing of both subjects in the auditory DMS task. A total of n = 12 sessions of testing are shown in Fig. 6A for subject 1 who had an average percent correct of 89% ± 1.34 SEM. Subject 2 completed n = 12 testing sessions with a mean of 74% (± 2.1% SEM) correct across both blocks of all sessions. We compared the performance for the post-ibotenic acid lesion sessions with the performance during the warm control sessions in a one-way ANOVA for each subject (Fig. 6B) and did not find a significant difference (subject 1, F(1, 22) = 0.624, p = 0.438; subject 2, F(1, 18) = 3.66, p = 0.0.716 (Fig. 6B).

3.5 Assessment of Gaze Drops During Task

In addition to the assessment of performance of the auditory working memory task we also examined the number of lost fixations throughout the session in each of the experiments (Cooing, Warm-Warm Control and Post-Lesion). During the AWM task a trial is initiated by the subject voluntarily fixating a central 4° white dot to begin a trial and then maintaining their gaze within a large viewing window

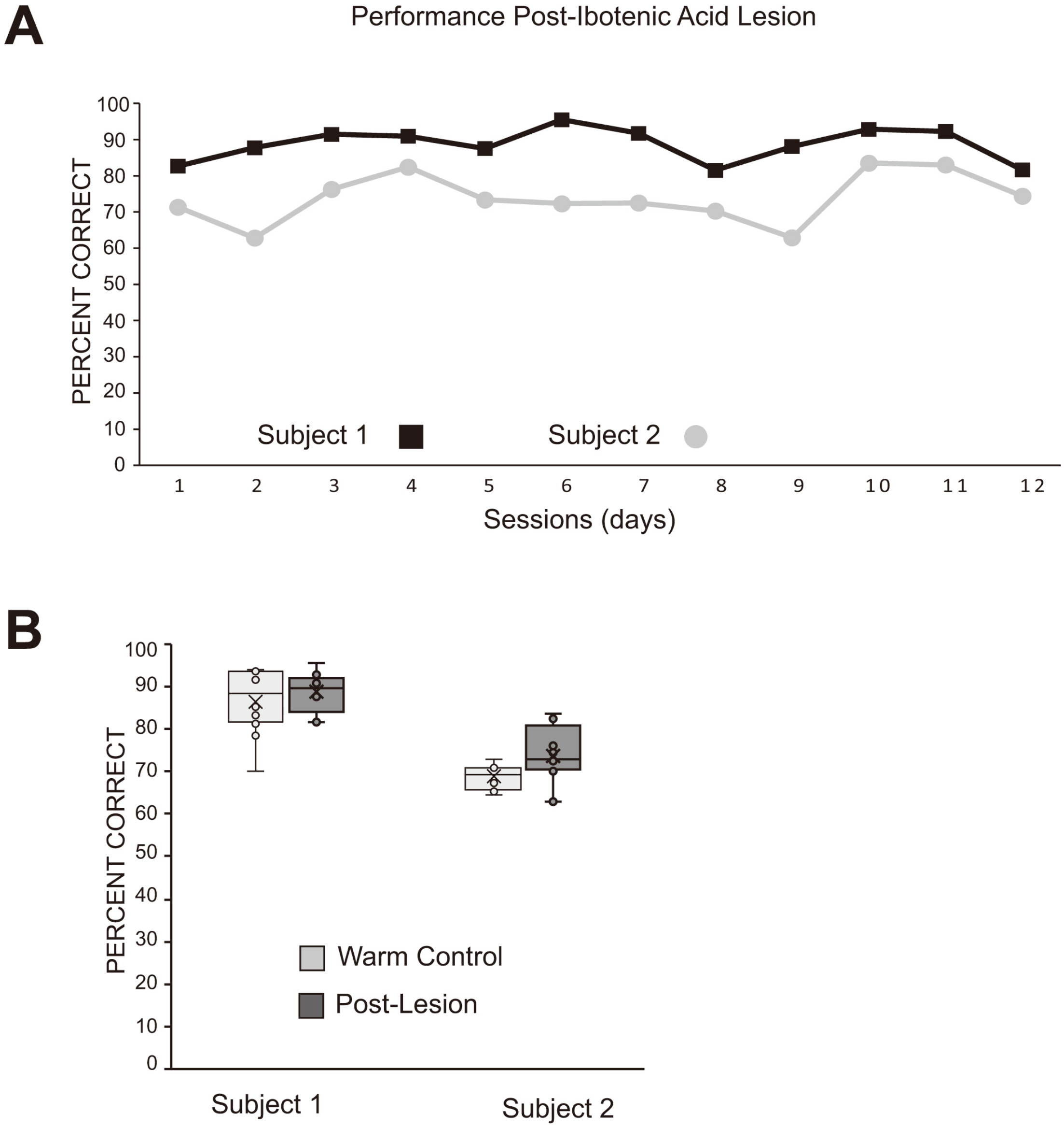

Fig. 6. Effects of ibotenic acid lesion in DLPFC on auditory DMTS performance. (A) Timeline of Auditory DMTS sessions conducted post-ibotenic acid injections into DLPFC are shown for subject 1 (black squares) and subject 2 (grey circles). (B) Average performance across all sessions post-ibotenic acid injection is plotted with the control performance in warm-warm sessions which was obtained previously. There was no significant change in overall accuracy after ibotenic acid lesions for either subject compared to control sessions.

demarcated on the monitor. This requirement helps the monkeys to maintain their attention to the task and makes the transition to visual stimulus paradigms easier. If a subject dropped their gaze out of the large window during a trial, before a response is made, the trial is aborted and restarted. Dropped fixations or gaze can be due to a number of factors but occur more often in the late part of the session, when the animal subjects are sated and are often interpreted as a loss of attention, motivation, or a sign of fatigue.

We compared the number of dropped gaze events during the cooling experiment in the early Warm period with dropped gaze events during the cooling inactivation period using a paired *t*-test. Subject 1 had an average of 10.25 (± 1.4 SEM) dropped gaze events in the pre-inactivation "warm" block versus 39.75 (± 6.6 SEM) lost fixations during the cortical cooling block. A paired *t*-test showed this increase of lost fixations/gaze was significant (t(11) = –4.64, $p < 0.001$, two-tailed). We used Cohens' *d* to estimate the effect size, using the mean difference of lost gaze events during the warm baseline minus the events during cooling, divided by the standard deviation of the differences. Cohens' *d* = –1.340 [CI: 12.116, –0.534]. This indicates a large effect with significantly more lost gaze events during the inactivation session than during the warm baseline period. Subject 2 had fewer overall lost gaze events in the cooling experiments with an average of 9.0 (± 2.2 SEM) in

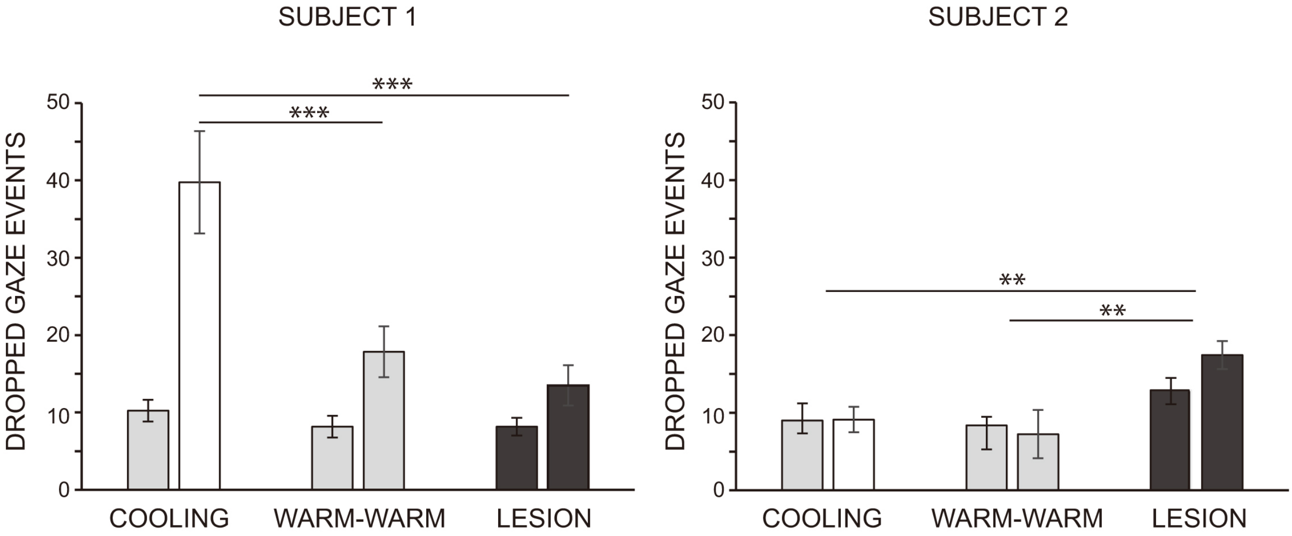

Fig. 7. Dropped Gaze Events. The mean and SEM of dropped gaze events which occurred in each of the two blocks for each experiment are shown for each subject. In subject 1, there was an increase in dropped gaze events in block 2 across all experiments and a significant increase in dropped gaze events during the cooling block of the cooling experiment. In subject 2, the number of total dropped gaze events was significantly increased in the post-lesion testing compared to the total in cooling and warm-warm experiments. Cooling block: white bar; Warm blocks: grey bars, Post lesion testing: Dark grey bars. Error bars are SEM. *** $p < 0.001$; ** $p < 0.01$.

the baseline warm block and 9.2 (± 1.6 SEM) in the cooling block. A paired *t*-test indicated no significant difference in the number of lost fixations during the pre-inactivation "warm" block compared to the cortical cooling inactivation block (t(5) = –0.062, $p = 0.95$, two-tailed).

The lost fixations by subject 1 during cooling may indicate an effect on attention by the cooling inactivation of DLPFC or could be due to a loss of motivation or an increase in fatigue during the second block of trials and not an effect of cooling. To examine this issue further a two-way ANOVA was performed separately on each subject, to evaluate the effects of type of experiment (Cooling: Warm-cool; Control: Warm-warm; post-lesion: warm-warm) and session block (First block-second block) on lost gaze events. The means and SEM are depicted in Table 2 below and in Fig. 7.

For subject 1, the results indicated a significant main effect for Experiment, F(2, 66) = 10.539, $p < 0.001$, partial $\eta^2 = 0.242$. There was also a significant main effect of block number, F(1, 66) = 29.865, $p < 0.001$, partial $\eta^2 = 0.312$. The number of gaze events for block 2 was significantly greater than gaze events in block 1 for all experimental conditions. There was a significant interaction of experiment and block number F(2, 66) = 7.512, $p = 0.0012$, partial $\eta^2 = 0.185$. The average dropped gaze events during the cooling block of the cooling experiment, 39.75 (± 6.0 SEM) was significantly greater than dropped gaze events in block 2 of the Control warm-warm experiment (Post-hoc Tukey test, $p < 0.001$) and the Post-lesion experiments (Post-hoc Tukey test, $p < 0.001$). The significant increase in dropped gaze events between the first and second blocks in all experiments suggests that a decrease in motivation or increase in fatigue might cause subject 1 to abort more trials in the second block. However, the significant interaction and post-hoc comparisons indicates that the inactivation of DLPFC with cortical cooling played a role in this effect in subject 1.

For subject 2, the two-way ANOVA results indicated a significant main effect for Experiment, F(2, 44) = 8.83, $p < 0.001$, partial $\eta^2 = 0.286$, but not for Block number, F(1, 44) = 0.508, $p = 0.480$, partial $\eta^2 = 0.011$. The interaction of experiment and block number was not significant F(2, 44) = 1.260, $p = 0.294$, partial $\eta^2 = 0.054$. Post-hoc pairwise comparisons with the Tukey test showed a significant increase in the post-lesion dropped gaze events compared to dropped gaze events in the Cooling experiment ($p = 0.014$) and the control warm-warm experiment ($p < 0.001$). Although neither subject demonstrated a significant change in task performance as a result of the cooling inactivation or the ibotenic acid lesions of the DLPFC, there were changes in behavioral events that suggest effects on motivation or attention which merit further study.

3.6 Localization of Ibotenic Acid Lesions

At the completion of testing the subjects were perfused and the prefrontal cortex was histologically processed and stained with thionin Nissl stain. Coronal sections through the prefrontal cortex were digitized using NeuroLucida (Microbrightfield, Inc.). Evidence of cell loss, degeneration and pyknotic nuclei were seen in the cortex of DLPFC, indicating the location of the ibotenic acid injections and the ensuing neuronal loss. This was demarcated on the Nissl

Table 2. Dropped gaze events: The means and SEM of dropped gaze events are shown for the first and second blocks of each experimental condition for each subject.

	Subject 1		Subject 2	
Experiment	Block 1	Block 2	Block 1	Block 2
Cooling	10.25 ± 1.4	39.75 ± 6.6	9 ± 2.20	9.12 ± 1.64
Warm-Warm	8.17 ± 1.4	17.83 ± 3.3	8.38 ± 1.1	7.25 ± 3.1
Post-lesion	8.2 ± 1.2	23.69 ± 2.6	12.91 ± 1.5	17.45 ± 1.6

stained NeuroLucida charted sections. The degeneration did not extend into the entire depths of the principal sulcus. These plots were overlaid on low-power photographs of the same Nissl sections in Adobe Illustrator and aligned with the location of DLPFC areas 8, 9, 46, and 46v in the rhesus monkey atlas NIH atlas – RED and [55]. A schematic of the extent of the lesions is depicted in Fig. 8 (Ref. [55]) for each subject with corresponding Nissl sections from the lesioned area. The targeted region of DLPFC was confirmed with some sparing of the depths of the principal sulcus.

4. Discussion

In the current study we examined the role of DLPFC in auditory working memory using a temporary inactivation by bilateral cortical cooling and permanent lesions with ibotenic acid. Temporary inactivation of DLPFC with cortical cooling did not significantly decrease performance of the auditory DMTS working memory task compared to performance during baseline in our two subjects. The lack of an effect on performance prompts the question of whether cortical cooling would cause an impairment in auditory working memory in another region or with a different task. The same cooling method was used in our laboratory to inactivate a different region, VLPFC. In that study [45], rhesus macaques performed an auditory and an audio-visual working WM while cortical cooling was used to temporarily inactivate VLPFC in the same way that the current study used cooling to inactivate DLPFC. Inactivation of VLPFC by cooling significantly decreased the performance of the auditory WM task [45], while cooling of DLPFC using similar methods in the current study, did not decrease auditory WM performance. This suggests that the technique is not an issue. Furthermore, the ibotenic acid lesions made in the current study, which were placed into the same DLPFC region as the cooling inactivation, also did not evoke a significant decrement in task performance. Thus, a role for DLPFC in auditory working memory is not clear, though its role in spatial working memory is well-known. In particular, performance of oculomotor delayed response (ODR) and spatial delayed response tasks has been found to be dependent on the activity and integrity of DLPFC [19,21,59]. Neurophysiological recordings during these visuo-spatial working memory tasks revealed DLPFC neurons with delay activity that represented the location of visual cues [17,19,59]. Inactivation of these neurons using cooling of DLPFC or lesions of DLPFC, impaired performance during visuo-spatial tasks [21,60]. The combined cooling inactivation and lesion of DLPFC which did not have a significant effect on auditory WM in the current study strengthen the hypothesis that regions other than DLPFC support nonspatial auditory working memory, including VLPFC which receives robust auditory afferents from the temporal lobe. These data support an argument for Domain Specificity [14] in nonhuman primates with DLPFC playing an essential role in visuo-spatial working memory and possibly auditory spatial processing [61,62] and VLPFC playing a role in feature based auditory discrimination.

While performance did not differ significantly in the normal "warm" trials compared to trials during the cortical cooling inactivation, our study has several limitations. While the use of a small number of subjects in studies utilizing non-human primates is common, the small sample size may underpower results. In neurophysiological studies multiple recordings of many neurons is typically done to confirm results. In the present study multiple sessions of cooling inactivation experiments and control experiments were done to increase the reliability of the results.

In addition, the auditory DMTS task used showed that performance was significantly worse during trial type 2, when subjects must inhibit the button-press responses during the presentation of the nonmatch stimulus and wait for the match stimulus. The error committed in trial type 2 was a "false alarm" button press i.e., an inability to inhibit the prepotent button press response during the nonmatch stimulus as subjects waited for the match stimulus and has been shown in prior studies using similar designs. A similar increase in type 2 errors has been shown previously [45]. A two-way ANOVA of temperature and trial type did not indicate that performance in trial type 2 was worsened during cooling inactivation in either subject.

4.1 Lateral Prefrontal Cortical Areas and Functional Specificity

DLPFC has been strongly linked with working memory and decision making in both human and animal studies. In nonhuman primate lesion and neurophysiology studies, DLPFC has been shown to play an important role in the processing and remembering of visual and visuospatial information [12,16,22,63]. The Domain Specificity theory suggests a segregation of prefrontal functional domains with each region having content-specific processing and storage mechanisms [12,64], across modalities. Support for

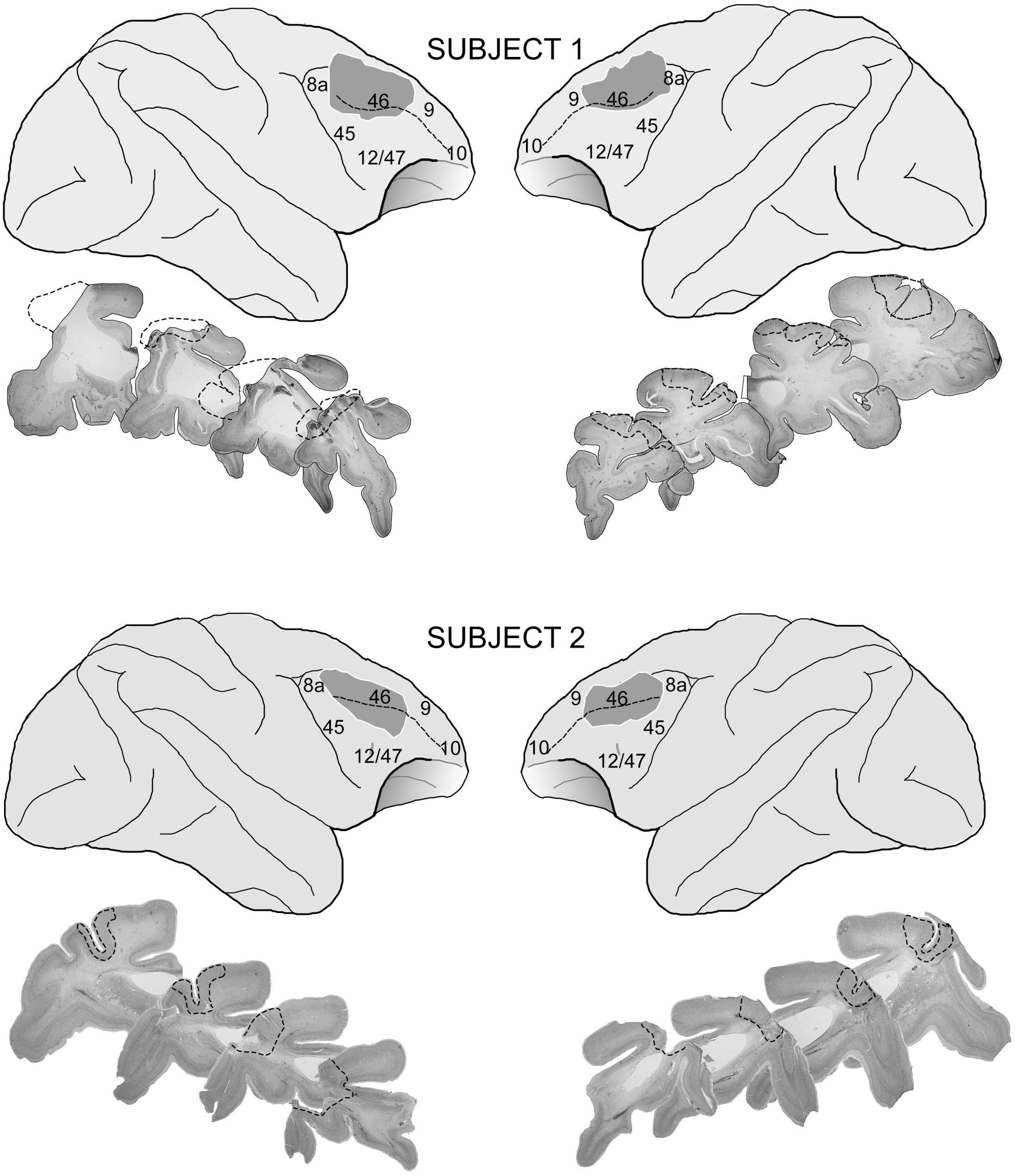

Fig. 8. Location of cooling region and ibotenic acid lesions in DLPFC. The location of the ibotenic acid lesions for subjects 1 and 2 are plotted on a brain atlas schematic RED, NIH Atlas [55]. Nissl-stained coronal sections through the DLPFC are shown below each corresponding subject's brain schematic and were examined and charted in NeuroLucida. Areas which showed cell loss and pyknotic nuclei were demarcated with dotted lines. In subject 1 the histological processing resulted in some tissue loss due to the ibotenic lesion which included the tissue within the dotted lines. Both cooling and ibotenic acid lesions failed to produce a deficit in the auditory discrimination task.

this theory comes from neurophysiological and lesion studies highlighting the importance of DLPFC in visuospatial working memory and examples of VLPFC neurons showing selectivity for visual features [16], including selectivity for faces [24,25]. Fewer studies have demonstrated the same dichotomy for the processing of auditory information.

Early analysis of the prefrontal cortex utilized lesions to determine functional contributions to visual and auditory nonspatial working memory. Cortical ablations that included portions of both dorsal and ventral parts of the lateral prefrontal cortex in Old World monkeys were made to assess its contribution to visual delayed response performance and auditory discrimination [27–30]. Gross and Weiskrantz [29] and later, Iversen and Mishkin [31] demonstrated that lesions of the principal sulcus impaired delayed response performance with visual memoranda, but it was only lesions, that included the inferior convexity, below the principal sulcus, that interfered with auditory discrimination. Later, neurophysiological recording of neurons in the lateral prefrontal cortex revealed task-related responses during auditory categorization [34,65], auditory working memory [32,33,66] and auditory decision making [67,68]. Neurophysiological recordings in macaque VLPFC which examined neural responses to complex sounds, demonstrated that neurons were responsive to features of species-specific vocalization stimuli [35,37,40,49,69].

In the current study, our goal was to investigate the role of DLPFC in auditory working memory since previous assessment of auditory and audiovisual working memory found that inactivation of VLPFC caused a significant decrease in performance [45]. The region of VLPFC that was inactivated in Plakke *et al*., (2015) [45] and which resulted in decreased AWM performance, included areas 12/47, 45 but also portions of 46v, which is often considered part of DLPFC. We therefore theorized that the VLPFC cooling effect could have been due to inclusion of area 46v. This hypothesis is also strengthened by the fact that the ventral portion of area 46 receives auditory cortical afferents from the rostral auditory parabelt and rostral auditory temporal cortex [70–72]. Furthermore, it has also been argued that the DLPFC plays a general role in many cognitive processes regardless of modality or type of information. However, the results of the current study demonstrated no significant performance decrement during AWM with transient or permanent DLPFC lesions that included areas of 46v on the lateral surface of the convexity. The present results therefore suggest different roles for DLPFC and VLPFC in non-spatial auditory cognition. Feature based auditory WM processing may rely less on DLPFC and more on VLPFC which receives a greater number of auditory afferents from belt, parabelt and auditory association areas [71,73,74]. These connections allow for the robust responses to complex communication calls and for integrated responses to face and vocal stimuli, that have been demonstrated in VLFPC [40,48,49]. Recordings made in VLPFC during the performance of an audiovisual nonmatch to sample task have shown that neurons demonstrate both sensory and context-modulated sensory responses [33]. Recordings made in the same DLPFC regions as the cooling experiments in the current study indicated that the majority of recorded neurons demonstrated significant firing rate changes to task events [38], rather than stimuli. Thus, a key difference is that VLPFC neurons appear to exhibit robust, stimulus-selective responses, to complex auditory stimuli while DLPFC exhibits task and context-related responses, which may be entwined with stimuli [9,39,40]. Additional studies with neurophysiological recordings performed simultaneously in both regions may yield clues to their specific roles in auditory cognition.

4.2 Behavioral Performance

Although ibotenic acid lesion and transient inactivation of DLPFC did not significantly decrease performance during auditory WM there was a significant increase in dropped gaze events in one subject during DLPFC cooling inactivation and in the second subject after ibotenic acid lesions of DLPFC. Dropping fixation or gaze during the task may occur due to several factors including a decrease in motivation or attention as well as fatigue. The fact that dropped gaze events were increased in all experiments in the second testing block suggests that fatigue or a loss of motivation is likely. For subject 2 the increase in dropped gaze events during the task after the lesion suggests the lesion had an effect on motivation or attentional factors. Performance during auditory discrimination paradigms is typically reduced compared to performance in visual working memory paradigms in the same subjects [38,45,57,75]. It is possible that the difficulty of the auditory discrimination task in the current study resulted higher distractibility, loss of motivation or loss of attention which was worsened by inactivation or lesions of DLPFC.

In accordance with the literature, we referred to our study as a working memory (WM) task. WM is thought to be an active process that requires attentional control and can allow for manipulation of the contents of working memory whereas short term memory (STM) is the simple storage of sensory information, a passive process [57]. Some have argued that nonhuman primates demonstrate passive STM but not active WM [57,76,77], and the false alarm errors seen in our study may support this idea.

In the current study and in previous investigations of auditory and audiovisual working memory [45], there was in increase in "false alarm" errors where subjects must reject a non-target stimulus by withholding or suppressing a button press on type 2 trials. This occurred in both the warm and cold sessions and was not related to the inactivation of DLPFC. The specific ability to inhibit a prepotent responses, referred to as a lack of cognitive control, is considered a hallmark of prefrontal cortical function [1]. Inhibitory control of prepotent responses has been explored extensively in a number of tasks, especially the Stroop task where it has been shown to involve several cortical regions including the anterior cingulate and DLPFC [78,79] though prepotent response disinhibition during trial type 2, was apparent in our subjects even within control sessions, without DLPFC inactivation.

In the auditory WM task used in the current study, the stimuli that were presented as samples, were "repeated" and used as matches in some trials and non-matches in others, to properly differentiate among stimulus and working memory effects on performance. This type of presentation increases the amount of proactive interference [57,80], making the task more difficult. Behavioral performance in auditory WM tasks [81] has been shown to be impaired by proactive interference which affects the evaluation of non-match test stimuli. Bigelow and Poremba [81] noted that during tasks with repeated stimuli and increased proactive interference, subjects are more likely to consider the test stimulus as a match, possibly due to its increased familiarity having recently been seen. They will treat the “nonmatch” as a “match” and press the button incorrectly. This could explain the larger number of errors in trial type 2 in our task, which requires subjects to withhold a press response when the second stimulus does not “match” the presented sample stimulus.

5. Conclusions

Our results showed that DLPFC transient inactivation with cortical cooling and ibotenic acid lesions did not impair performance on an auditory WM task. Our data suggests that DLPFC and VLPFC may play different roles in auditory working memory. The well-known role that DLPFC plays in visuo-spatial WM may carry over into auditory spatial WM as well, although this has not been clearly demonstrated. In contrast, the role that VLPFC plays in object-feature WM for both auditory and visual objects has been clearly defined in studies of nonhuman primates [22,24,33,35]. Future neurophysiological studies which can examine neuronal responses in DLFC and VLPFC simultaneously, may disentangle the different roles that these regions play in auditory cognition.

Availability of Data and Materials

All datasets reported in this study are available for scientific research purposes upon request.

Author Contributions

BP designed the experiments, supervised, and participated in experimental procedures, performed data acquisition and data analysis, wrote initial drafts of the manuscript, critically reviewed and revised the manuscript. KSK performed the experimental procedures, performed data acquisition and analysis, and critically reviewed manuscript drafts. CL participated in experimental procedures, performed data analysis, and created graphs and illustrations for the manuscript, and critically reviewed drafts of the manuscript. LMR designed the experiments, supervised the experimental procedures, performed data analysis, critically reviewed and revised drafts of the manuscript, reviewed and revised all content for final approval. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All procedures were performed in accordance with the NIH’s Guidelines for the Care and Use of Animals in Research and were approved by the University of Rochester Committee on Animal Resources, Vivarium and the Division of Comparative Medicine (101503 / 2000-135), which are fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) and comply with state law, federal statute and NIH policy.

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Conflict of Interest

The authors declare no conflict of interest.

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