

Impaired Visual Search in Rats Reveals Cholinergic Contributions to Feature Binding in Visuospatial Attention

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The visual search task established the feature integration theory of attention in humans and measures visuospatial attentional contributions to feature binding. We recently demonstrated that the neuromodulator acetylcholine (ACh), from the nucleus basalis magnocellularis (NBM), supports the attentional processes required for feature binding using a rat digging-based task. Additional research has demonstrated cholinergic contributions from the NBM to visuospatial attention in rats. Here, we combined these lines of evidence and employed visual search in rats to examine whether cortical cholinergic input supports visuospatial attention specifically for feature binding. We trained 18 male Long-Evans rats to perform visual search using touch screen-equipped operant chambers. Sessions comprised Feature Search (no feature binding required) and Conjunctive Search (feature binding required) trials using multiple stimulus set sizes. Following acquisition of visual search, 8 rats received bilateral NBM lesions using 192 IgG-saporin to selectively reduce cholinergic afferentation of the neocortex, which we hypothesized would selectively disrupt the visuospatial attentional processes needed for efficient conjunctive visual search. As expected, relative to sham-lesioned rats, ACh-NBM-lesioned rats took significantly longer to locate the target stimulus on Conjunctive Search, but not Feature Search trials, thus demonstrating that cholinergic contributions to visuospatial attention are important for feature binding in rats.

Keywords: acetylcholine, basal forebrain, nucleus basalis magnocellularis (NBM)

Introduction

The fundamental cognitive process known as feature binding refers to the attention-dependent mechanism by which different features of an object are integrated to form a unified neural representation. Using a digging-based feature binding task, we have previously shown that the neuromodulator acetylcholine (ACh) and cholinergic neurons in the nucleus basalis magnocellularis (NBM) of the basal forebrain are necessary for efficient feature binding in rats, and our cross-species work, in rats and humans, suggests that ACh supports the attentional processes needed for efficient feature binding (Botly and De Rosa 2007, 2008, 2009). However, in the human cognitive literature, the standard test of feature binding is visual search, which measures visuospatial attention and established the feature integration theory of attention (Treisman and Gelade 1980; Corbetta et al. 1995; Treisman 1998; Nobre et al. 2003).

In the visual search paradigm, participants must locate a visual target embedded in an array of distractor stimuli. On Feature

Search trials, feature binding is not required because participants can look for a single feature (e.g., look for the color green to find a green apple in a basket of red apples and red pears). On Conjunctive Search trials, feature binding is required because participants must look for a conjunction of 2 features (e.g., look for the color green and the shape of an apple to find a green apple in a basket of red apples, red pears, and green pears). During Feature Search, the demand on attention is minimal as targets “pop out” regardless of the number of distractors present, while during conjunctive search, an attentionally demanding serial-like search for the target is necessary as evidenced by a significant increase in latency to locate the target stimulus as the number of distractor stimuli is increased in human participants (Treisman and Gelade 1980; Treisman 1998).

There has been a wealth of important research demonstrating cortical cholinergic contributions from the NBM to visuospatial attention in rats using pharmacology, brain lesioning, in vivo microdialysis, and electrophysiology (Gill et al. 2000; Himmelheber et al. 2000; Passetti et al. 2000; Arnold et al. 2002; McGaughy et al. 2002; Lehmann et al. 2003; Sarter et al. 2003; Broussard et al. 2006, 2009; Parikh et al. 2007; Harati et al. 2008; Parikh and Sarter 2008). The dominant paradigm used in this line of research has been the 5-choice serial reaction time task (5-choice), which assesses visuospatial attention by requiring sustained attention to 5 potential physical locations in which a target light stimulus could appear. In this task, attention is taxed by limiting sensory information with decreased target stimulus durations (McGaughy et al. 2002; Lehmann et al. 2003; Harati et al. 2008). In contrast, in the visual search task, visuospatial attention is selectively taxed during Conjunctive Search trials by presenting competing distractor stimuli that contain the same features as the target stimulus such that feature binding is required for target detection. Although research using tasks like the 5-choice has established an important functional relationship between cortical ACh and visuospatial attention, it is still important to ask whether one function of cholinergic contributions to visuospatial attention is its importance to feature binding.

Over the decades, visual search has been extensively tested in nonhuman animals such as pigeons and primates (Blough 1984; Cook 1992; Vreven and Blough 1998; Balan et al. 2008; Nothdurft et al. 2009), but it has never been successfully implemented in rats. Moreover, unlike our digging-based feature binding task and the classic 5-choice task, visual search examines the focal attentional resources directed to one spatial location to bind features in the presence of direct competition from distractors also present in the visual display. This strict test of focal attention was not possible with our digging-based task where the competition from distractor stimuli was

indirect and associative or with the classic 5-choice task where there is no stimulus competition present. Hence, we designed a rodent analog of the visual search paradigm using touch screen-equipped operant chambers to test our hypothesis that one important function of cholinergic contributions to visuospatial attention is its support of feature binding. Following acquisition of the visual search task, we bilaterally lesioned the NBM of rats using the cholinergic-selective immunotoxin, 192 IgG-saporin. We predicted that a reduction of cortical cholinergic afferentation would disrupt visuospatial attention and result in less efficient visual search such that ACh-NBM-lesioned rats would take longer than sham-lesioned rats to locate the target stimulus on Conjunctive Search, but not Feature Search, trials.

Materials and Methods

Participants

The participants were 20 experimentally naïve male Long-Evans rats (Charles River, Montreal, Quebec) aged 6 weeks and weighing 195–225 g at the start of the experiment. Rats were housed individually in 45 cm long × 25 cm wide plastic tub cages with food available ad libitum. The vivarium was temperature and humidity controlled. Rats were maintained on a reversed 12 h light–12 h dark cycle (lights off at 8 AM), and training was conducted during the dark phase of the light:dark cycle between the hours of 10 AM and 5 PM, 6–7 days a week. One week prior to the start of the experiment, rats were handled for 15 min per day for 7 days with water available ad libitum. Rats were water restricted 24 h before the start of training. Throughout the experiment, rats received water during training (0.05 mL per correct response) and were given ad libitum access to water for 20–30 min each day following training. This study was approved by the University of Toronto's Institutional Animal Care Committee.

Apparatus

Four custom-made operant chambers (MED Associates Inc.) equipped with touch screens (ELO) were used. Figure 1 illustrates an overhead view of a single touch screen-equipped operant chamber (41.2 × 41.2 × 29.2 cm [l × w × h]) housed inside a sound- and light-attenuating enclosure (74 × 60.5 × 60 cm) made of wood and equipped with a fan to provide ventilation and masking noise. The right and left operant chamber walls and ceiling were made of clear Plexiglas, and the right wall was hinged creating an interior door for access to the chamber. The front and rear walls were made of stainless steel, and the floor of the chamber was composed of steel rods (5 mm diameter) spaced 1.1 cm apart horizontally. The bottom of the touch screen was level with the floor of the chamber. The touch screens were 24.7 cm wide × 18.5 cm high and positioned in the center of the front wall of the chamber with 7.9 cm on either side. Touch screens were inset 1.6 cm from the front wall of the chamber. A rectangular water well (5 × 5 × 5 cm) was located in the center of the rear wall of the chamber. A water pump equipped with a 25 mL syringe dispensed 0.05 mL of water at a time. A circular white light 2.5 cm in diameter was located 2.2 cm above the water well and acted as a cue to the availability of water. Each operant chamber contained 3 pairs of infrared (IR) emitters and detectors. Two pairs were located on the right and left walls of the chamber and were positioned 4 cm above the floor to detect movement. One of these pairs was located 5.5 cm from the back wall of the chamber (back IR beam) and the other was equidistant between the rear and the front wall of the chamber (middle IR beam). The third pair was positioned on either side of the water well (water IR beam) to detect drinking. Each operant chamber was equipped with a speaker and an IR webcam mounted to the ceiling to allow real-time monitoring and video recording of rats' performance. A client CPU controlled all the inputs and outputs of the chamber via MED-PC IV (MED Associates Inc.) and proprietary software (Wojtek Grabski) and another CPU controlled the IR webcam. One server computer, located outside of the testing room, controlled all 4 client CPUs via a proprietary control interface (Wojtek

Grabski) and MED-PC IV software (MED Associates Inc.). A second computer, also located outside of the testing room, monitored and recorded the output of all 4 IR webcams via Active WebCam (PY Software) software.

Visual Stimuli

A set of black-and-white computer-generated stimuli was created: 1 target stimulus (white square) and 3 distractor stimuli (black square, white triangle, and black triangle). Each stimulus measured 5 × 5 cm (Fig. 2). We did not want target stimulus identity to contribute to individual variability in visual search task performance and thus, we did not counterbalance the identity of the target stimulus. We chose to use the white square as the target stimulus for all rats throughout the entire experiment. Accordingly, the rats' task never changed across trials and only the context in which the target was presented changed as the distractors in the display dictated whether a Feature or Conjunctive search was required.

Pretraining Procedures

Habituation

Prior to water restriction, each rat was exposed to the inside of an operant chamber for 15 min per day for 2 days.

Light-Water Training

Twenty-four hours following the start of water restriction, rats began light-water training using a variable-interval 60-s (15 s variance) schedule of reinforcement. The light above the water well indicated the availability of water during which rats could insert their nose into the water well to break the IR beam and activate the water pump to receive a reward of 0.05 mL of water. Rats received 30 min sessions per day until criterion performance of breaking the IR beam in the water well on average less than 2 s after the onset of the water light occurred within a session for all rats.

Touch-Light-Water Training

Rats were required to touch the touch screen with their nose or front paws to activate the water light and then break the water IR beam to receive a reward of 0.05 mL of water. To discourage rats from directing their touches to the corners of the touch screen, a 22.9 cm wide × 15.2 cm high central response area was used during all phases of the experiment. Surrounding the central response area was 1.1 cm of nonresponsive touch screen on either side and 1.59 cm on the top and bottom. An intertrial interval (ITI) of 2 s was employed, which was approximately the same length of time spent by rats in the water well following the offset of the water light. Rats received 40 min of training or 72 trials per session (whichever came first) until criterion performance of touching the touch screen on average less than 10 s from the offset of the ITI occurred within a session for all rats.

Single-Stimulus Training

Rats were trained to touch a white square stimulus presented in the center of the touch screen to activate the water light and then break the water IR beam to receive 0.05 mL of water. The area of the touch screen surrounding the centrally presented stimulus was inactivated to facilitate training, and thus, incorrect responses were not possible. The stimulus remained on the screen until a response was made, and it disappeared immediately following a response. An ITI of 2 s was employed. In order for a trial to be initiated, the back IR beam had to be broken for 2 s, but the water IR and middle IR beams had to be intact during this time interval. Hence, rats had to be at the back of the chamber for a stimulus to be presented, which ensured that rats did not impulsively run up to the touch screen before a stimulus was presented. As soon as the IR requirements for trial initiation were met, a 10 KHz warning tone was presented for 2 s. The stimulus was presented 1 s after the onset of the warning tone. Presentation of the warning tone was intended to signal to the animal that a stimulus was about to be presented. If the middle or water-well IR beams were broken during presentation of the first second of the warning tone, no stimulus was presented, and rats had to break the back IR beam again to

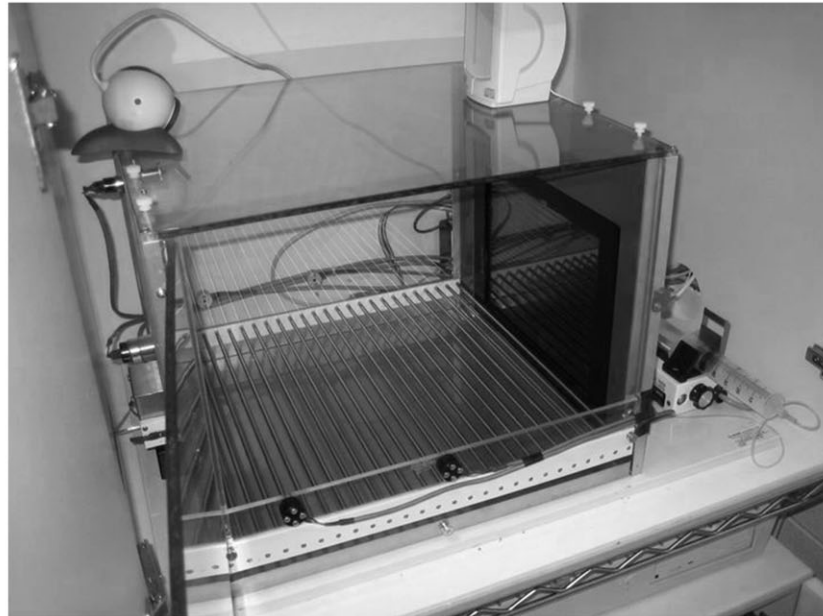
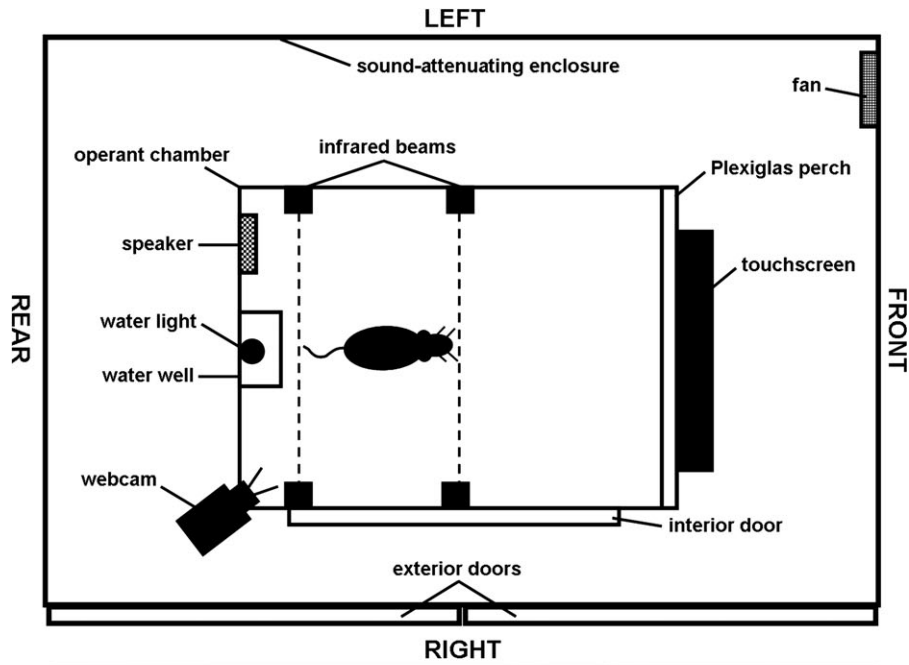


Figure 1. Schematic overhead view of a touch screen-equipped operant chamber housed inside a sound- and light-attenuating enclosure as well as a photograph.

initiate the next trial. Rats received 40 min of training or 72 trials per session (whichever came first) until criterion performance of touching the stimulus on average less than 10 s from stimulus onset occurred within a session for all rats.

Follow-Stimulus Training

The touch screen was divided up into a 2×4 grid, and rats were trained to touch the white square stimulus which could appear in any one of the 8 locations on the touch screen (4 locations on the top half and 4 locations on the bottom half of the touch screen) to activate the water light and then break the water IR beam to receive a reward of 0.05 mL of water. To facilitate the touching of stimuli presented on the top half of the touch screen, a clear Plexiglas perch $30 \times 0.5 \times 1.0$ cm ($l \times w \times h$) was adhered with Velcro to the front of the touch screen so that rats could rear and place their paws onto it when reaching for stimuli, thus preventing them from inadvertently touching the lower half of the touch screen in the process. The perches were positioned

such that they did not obscure the view of stimuli presented on the lower half of the touch screen. The perches were left in the chambers for the remainder of the experiment. The same general trial procedures as those discussed above were used. All areas of the touch screen other than the location of the stimulus were inactivated, and thus, incorrect responses were not possible. Rats received 72 trials per session, and the location of the stimulus was counterbalanced within a session such that the stimulus was presented 9 times in each location per session in a pseudorandom order. Training continued until criterion performance of touching the stimulus on average less than 10 s from stimulus onset occurred within a session for all rats.

Simultaneous-Discrimination Training

The touch screen was divided up into a 2×2 grid (2 stimulus locations on the top half and 2 stimulus locations on the bottom half of the touch screen), and rats were trained to touch the white square stimulus, from now on referred to as the “target” stimulus, when presented along with

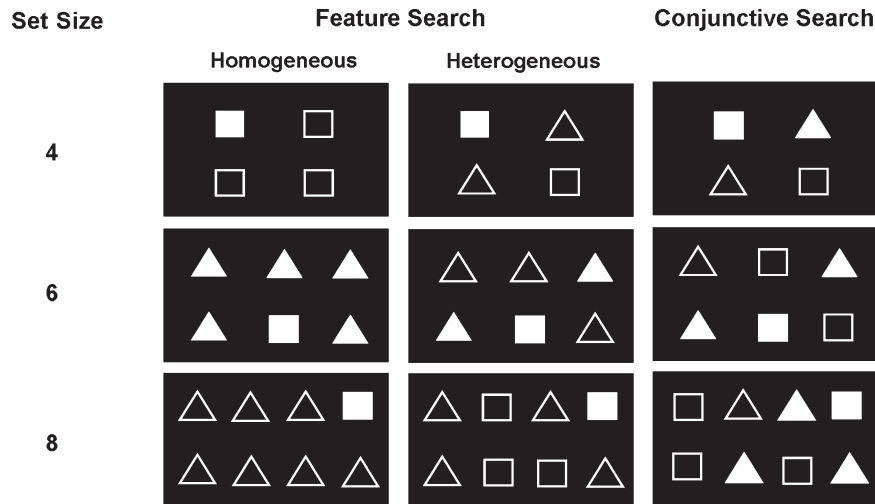


Figure 2. Illustration of the 2 different trial types, Feature Search and Conjunctive Search, and the 3 different stimulus set sizes (4, 6, and 8 stimuli) used in the visual search task. The target stimulus was always the white square, and rats were rewarded with access to water for touching the target. Each visual search task session comprised a total of 144 trials counterbalanced for target and distractor positions, trial type (Feature Search or Conjunctive Search), and stimulus set size. There were 2 different types of Feature Search trials, homogeneous and heterogeneous. On homogeneous trials, all distractors presented were identical, while on heterogeneous trials, the distractors presented were not identical. One type of heterogeneous Feature Search trial required discrimination of the target from the distractors based on pattern (black or white) and the other type required discrimination based on shape (square or triangle).

1 of 3 distractor stimuli (black square, white triangle, and black triangle). The same general trial procedures as those discussed above were used. Rats first received training using only the bottom 2 locations of the touch screen. All areas of the touch screen other than the locations of the 2 stimuli (1 target and 1 distractor) were inactivated. Rats received 72 trials per session and the location of the target stimulus was counterbalanced for left/right position within a session, and each of the 3 distractors was presented 24 times within a session in a pseudorandom order. The stimuli remained on the touch screen until a response was made. If rats made an incorrect response by touching the distractor stimulus instead of the target stimulus, both stimuli disappeared immediately from the screen following a response, and rats received a 10-s time-out period during which the entire touch screen turned white to signal a mistake. If rats made a correct response by touching the target stimulus and not the distractor stimulus, this activated the water light and rats could then break the water IR beam to receive a reward of 0.05 mL of water. Following an incorrect response, rats received a correction trial whereby the exact same trial was presented until a correct response was made.

Correction trials continued to be implemented until rats achieved 2 sessions during which a criterion level of performance was met. Criterion was defined as 18/20 correct responses anywhere within the 72-trial session. Once rats achieved 2 sessions of criterion-level performance without the assistance of correction trials, they moved onto simultaneous-discrimination training using only the top 2 stimulus locations of the touch screen. The same trial and counterbalancing procedures as those discussed above were employed. Following successful criterion-level performance with and then without the assistance of correction trials using only the top 2 stimulus locations of the touch screen, rats moved onto simultaneous-discrimination training using both the bottom and the top stimulus locations. Thus, the target and distractor stimuli appeared together equally often on either the top half (36 times) or the bottom half (36 times) of the touch screen within each 72-trial session in a pseudorandom order. The same trial and counterbalancing procedures as those discussed above were employed. Training continued until criterion-level performance with and without the assistance of correction trials was attained.

Visual Search Training Procedures

It was important to keep the target stimulus on the screen until the rats located it, so there was no limit on how long the stimulus would remain on the screen. This is comparable to how visual search is implemented

with human participants. Visual search is expressed in terms of the response time to correct target location in humans (Treisman and Gelade 1980; Treisman 1998), thus, we recorded both accuracy and latency to respond in rats. It is also important to note that in order to keep the size of the stimuli large enough for rats to accurately discriminate, our largest stimulus set size comprised only 8 stimuli, which is much smaller than the set sizes of approximately 20 stimuli typically employed with human participants (Nobre et al. 2003).

Visual Search Set-Size 4 Training

The touch screen was divided up into a 2 × 2 grid (2 stimulus locations on the top half and 2 stimulus locations on the bottom half of the touch screen), and rats were trained to touch the target stimulus (white square) when presented along with 3 distractor stimuli (black square, white triangle, and black triangle). Rats received 72 trials per session, half of which were Feature Search and the remaining half Conjunctive Search trials presented in a pseudorandom order. For the 36 Feature Search trials, the target stimulus location was counterbalanced for position, with each of the 4 possible positions repeated 9 times per session in a pseudorandom order. On every Feature Search trial, the target stimulus was presented in 1 of the 4 possible stimulus locations and the remaining 3 stimulus locations were occupied by 1 of the 3 distractor stimuli. Each distractor stimulus was repeated 12 times per session in a pseudorandom order. For the 36 Conjunctive Search trials, the target stimulus location was counterbalanced for position, with each of the 4 possible positions repeated 9 times per session in a pseudorandom order. On every Conjunctive Search trial, the target stimulus was presented in 1 of the 4 possible stimulus locations and the remaining 3 stimulus locations were occupied by 1 of each of the 3 distractor stimuli, counterbalanced for position within a session. Training continued until criterion-level performance was attained with and then without the assistance of correction trials. Criterion was defined as 18/20 correct responses anywhere within the 72-trial session. The same general trial procedures as those discussed above were employed. Throughout Visual Search Set-Size 4 training and for the remainder of the experiment, rats were always allowed 2 touches of the touch screen to make a correct response. This was employed to ensure that rats did not become frustrated during sessions as they were prone to inadvertently touching adjacent distractor stimuli while in the process of touching the target stimulus. However, on trials in which rats touched the target stimulus on its first touch, this first touch was rewarded. Only on trials in which a rat made an incorrect touch first did they receive a second chance to confirm their choice.

Visual Search Set-Size 6 Training

The touch screen was divided up into a 2×3 grid (3 stimulus locations on the top half and 3 stimulus locations on the bottom half of the touch screen), and rats were trained to touch the target stimulus (white square) when presented along with 5 distractor stimuli (black square, white triangle, and black triangle). Rats received 72 trials per session, half of which were Feature Search and the remaining half Conjunctive Search trials presented in a pseudorandom order. For the 36 Feature Search trials, the target stimulus location was counterbalanced for position, with each of the 6 possible positions repeated 6 times per session in a pseudorandom order. On every Feature Search trial, the target stimulus was presented in 1 of the 6 possible stimulus locations and the remaining 5 stimulus locations were occupied by 1 of the 3 distractor stimuli. Each distractor stimulus was repeated 12 times per session in a pseudorandom order. For the 36 Conjunctive Search trials, the target stimulus location was counterbalanced for position, with each of the 6 possible positions repeated 6 times per session in a pseudorandom order. On every Conjunctive Search trial, the target stimulus was presented in 1 of the 6 possible stimulus locations and the remaining 5 stimulus locations were occupied by the distractor stimuli, counterbalanced for position within a session. Because there were 5 stimulus locations for distractor stimuli to occupy, but only 3 different distractor stimuli, 1 of the distractor stimuli had to occupy 2 stimulus locations on each Conjunctive Search trial, while the others occupied 1 stimulus location. The selection of distractor stimuli for occupying 1 or 2 stimulus locations on Conjunctive Search trials was pseudorandomized within a session. The same general trial procedures as those discussed above were employed. Training continued until the criterion-level performance was attained with and then without the assistance of correction trials.

Visual Search Set-Size 8 Training

The touch screen was divided into a 2×4 grid (4 stimulus locations on the top half and 4 stimulus locations on the bottom half of the touch screen), and rats were trained to touch the target stimulus (white square) when presented along with 7 distractor stimuli (black square, white triangle, and black triangle). Rats received 96 trials per session, half of which were Feature Search and the remaining half Conjunctive Search trials presented in a pseudorandom order. For the 48 Feature Search trials, the target stimulus location was counterbalanced for position, with each of the 8 possible positions repeated 6 times per session in a pseudorandom order. On every Feature Search trial, the target stimulus was presented in 1 of the 8 possible stimulus locations and the remaining 7 stimulus locations were occupied by 1 of the 3 distractor stimuli. Each distractor stimulus was repeated 16 times per session in a pseudorandom order. For the 48 Conjunctive Search trials, the target stimulus location was counterbalanced for position, with each of the 8 possible positions repeated 6 times per session in a pseudorandom order. On every Conjunctive Search trial, the target stimulus was presented in 1 of the 8 possible stimulus locations and the remaining 7 stimulus locations were occupied by the distractor stimuli, counterbalanced for position within a session. Because there were 7 stimulus locations for distractor stimuli to occupy, but only 3 different distractor stimuli, 1 of the distractor stimuli had to occupy 3 stimulus locations on each Conjunctive Search trial, while the others occupied 2 stimulus locations. The selection of distractor stimuli for occupying 2 or 3 stimulus locations on Conjunctive Search trials was pseudorandomized within a session. The same general trial procedures as those discussed above were employed. Training continued until the criterion-level performance was attained with and then without the assistance of correction trials.

Visual Search All Set-Sizes Training

Rats received 144 trials per session, half of which were Feature Search and the remaining half Conjunctive Search trials presented in a pseudorandom order. Of the 72 Feature Search trials per session, 24 were Feature Search Set-Size 4 trials, 24 were Feature Search Set-Size 6 trials, and 24 were Feature Search Set-Size 8 trials, presented in a pseudorandom order. Both target stimulus location and distractor stimuli were counterbalanced within each set size of Feature Search trials. Of the 72 Conjunctive Search trials per session, 24 were

Conjunctive Search Set-Size 4 trials, 24 were Conjunctive Search Set-Size 6 trials, and 24 were Conjunctive Search Set-Size 8 trials, presented in a pseudorandom order. Both target and distractor stimuli locations were counterbalanced within each set size of Conjunctive Search trials. Unlike the 4-, 6-, and 8-Stimulus Set-Size training sessions, in this all set-size training, correction trials were not employed. The same general trial procedures as those discussed above were employed. To establish a stable level of performance prior to advancing to the final visual search task, rats received 12 Visual Search All Set-Sizes training sessions.

Visual Search Task

The final version of the visual search paradigm detailed below was the last training program given to rats prior to surgery and was subsequently used to assess rats' visual search performance following surgery. Figure 2 illustrates the different trial types and stimulus set sizes of the visual search task. To establish a stable level of performance prior to surgery, rats received 12 sessions of training on the visual search task, which consisted of 144 trials per session, half of which were Feature Search and the remaining half Conjunctive Search trials presented in a pseudorandom order. Of the 72 Feature Search trials per session, 24 were Feature Search Set-Size 4 trials, 24 were Feature Search Set-Size 6 trials, and 24 were Feature Search Set-Size 8 trials, presented in a pseudorandom order. To equate for the heterogeneity of the Conjunctive Search displays, we introduced heterogeneous Feature Search trials in which the distractor stimuli presented were not identical. Of the 24 Feature Search Set-Size 4 trials, half were homogeneous (3 identical distractors presented) and half were heterogeneous (2 black triangles and 1 black square presented or 2 black triangles and 1 white triangle presented). Of the 24 Feature Search Set-Size 6 trials, half were homogeneous (5 identical distractors presented) and half were heterogeneous (3 black triangles and 2 black squares presented or 3 black triangles and 2 white triangles presented). Of the 24 Feature Search Set-Size 8 trials, half were homogeneous (7 identical distractors presented) and half were heterogeneous (4 black triangles and 3 black squares presented or 4 black triangles and 3 white triangles presented). One of the 2 types of heterogeneous Feature Search trials required discrimination of the target from the distractors based on pattern (black or white) and the second based on shape (square or triangle). Both target and distractor stimuli locations were counterbalanced within each set size of Feature Search trials. Of the 72 Conjunctive Search trials per session, 24 were Conjunctive Search Set-Size 4 trials, 24 were Conjunctive Search Set-Size 6 trials, and 24 were Conjunctive Search Set-Size 8 trials, presented in a pseudorandom order. Both target and distractor stimuli locations were counterbalanced within each set size of Conjunctive Search trials. The same general trial procedures as those discussed above were employed. Correction trials were not employed.

Surgery

Of the 20 rats, 2 did not successfully acquire the pretraining tasks and were removed from the study. The remaining 18 rats were assigned to 1 of 2 surgical groups, sham-lesioned ($n = 8$) or ACh-NBM-lesioned ($n = 10$) equating the groups for presurgical visual search performance based on accuracy and latency to locate the target stimulus on correct trials. Surgeries were performed under aseptic conditions. Rats were anesthetized with isoflurane (approximate maintenance dose was 2% with 1 L/min of oxygen). A subcutaneous (s.c.) injection of the analgesic buprenorphine (0.03 mg/kg) and an intraperitoneal (i.p.) injection of atropine (0.05 mg/kg) were delivered immediately prior to surgery, the latter of which served to prevent fluid buildup in the lungs. Stereotaxic coordinates for NBM lesioning and dose of immunotoxin were the same as those employed in our crossmodal feature binding study (Botly and De Rosa 2009). Four 1.0 mm holes were drilled at the following stereotaxic coordinates relative to bregma and the surface of the skull (Paxinos and Watson 1998): anterior NBM: anterior/posterior (A/P) -0.8 mm, medial/lateral (M/L) ± 2.5 mm, and dorsal/ventral (D/V) -8.2 mm; posterior NBM: A/P -1.6 mm, M/L ± 2.5 mm, and D/V -7.6 mm. There were a total of 4 intraparenchymal injection sites, 2 per hemisphere, of 0.2 μ L sterile 0.1 M (pH 7.4) phosphate-buffered saline (sham lesion) or 0.2 μ g/ μ L 192 IgG-saporin (Advanced Targeting Systems, San Diego, CA, lot# 41-105) dissolved in sterile 0.1 M (pH 7.4) phosphate-buffered saline through a 26-gauge Hamilton syringe at

0.1 $\mu\text{L}/\text{min}$. The needle was left in place for 3 min after each injection. The body temperature of each rat was maintained with a homeothermic blanket throughout the surgery. After the injections were complete, a small piece of sterile hemostatic gelfoam was applied over the exposed skull to control any bleeding and the incision was closed with staples. Eutectic Mixture of Local Anesthetic topical analgesic ointment (2.5% lidocaine and 2.5% prilocaine) was liberally applied over the staples. To prevent dehydration, rats received warmed normal saline (0.9% NaCl; 2 mL/100 g body weight; s.c.) immediately postsurgery. All rats received a minimum of 14 days of recovery with ad libitum food and water before being water restricted for subsequent testing.

Postsurgical Testing Procedures

Using the same target and distractor stimuli as those employed prior to surgery, rats received 12 visual search testing sessions identical to the final test described above (visual search task) to establish a stable level of postsurgical performance. Experimenters were blind to the surgical group of the animals.

Histological Analyses

Rats were deeply anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and transcardially perfused with approximately 150 mL of ice-cold normal saline followed by approximately 150 mL of ice-cold 4% paraformaldehyde. Brains were extracted and immediately postfixed in 4% paraformaldehyde for 2 h at 4 °C and then transferred to a solution of 20% sucrose in phosphate-buffered saline (0.1 M; pH 7.4) and stored for 2 weeks at 4 °C. Brains were sectioned at a thickness of 60 μm using a cryostat equipped with a freezing-sliding microtome (Leica Microsystems, Canada). Adjacent sections were used for staining for acetylcholinesterase (AChE) histochemistry, choline acetyltransferase (ChAT) immunohistochemistry, and parvalbumin immunohistochemistry. AChE histochemistry was carried out according to the methods of Paxinos and Watson (1998) and was used to confirm cholinergic fiber loss in target neocortical structures of the NBM. ChAT and parvalbumin immunohistochemistry were carried out according to the methods of Botly and De Rosa (2009) to assess the extent of cholinergic and GABAergic cell body loss in the NBM and medial septum/vertical limb of the diagonal band of Broca (MS/VDB), respectively. After completion of all histological assays, brain slices were mounted on slides, dehydrated, and cleared using an ascending ethanol and xylene series, coverslipped with the histological mountant distyrene plasticizer xylene and examined under a Leica light microscope (DM4000B, Ontario, Canada).

Histological Quantification

Cell Counting

ChAT- and parvalbumin-immunoreactive cells were counted bilaterally in the NBM and MS/VDB as delineated by Paxinos and Watson (1998) for each rat across brain sections 300 μm apart using a Leica light microscope (DM4000B, Ontario, Canada) and Openlab image analysis software (Quorum Technologies, Ontario, Canada). For the NBM, cell counts were taken from brain sections at approximately the following A/P coordinates relative to bregma: A/P = -0.84, -1.32, and -1.56 mm. The rectangular outlines superimposed on the rat brain coronal schematics depicted in Figure 4 (Paxinos and Watson 2007) delineate the NBM cell-counting frames used for histological quantification. For the MS/VDB, approximately the following A/P coordinates relative to bregma were used for cell counting: A/P = +0.84, +0.72, and +0.60 mm. The rectangular outlines superimposed on the rat brain schematics depicted in Figure 5 (Paxinos and Watson 2007) delineate the MS/VDB cell-counting frames used for histological quantification. ChAT- and parvalbumin-immunoreactive cells were easily identifiable and characterized by relatively large cell bodies with several extending dendrites, and we only counted cells that were well distinguishable (i.e., well-defined borders) from the background.

AChE Densitometry

To quantify the reduction of cortical cholinergic input induced by our NBM cholinergic lesions, estimates of optical density in the frontal and

parietal cortices and hippocampus were obtained from photomicrographs of AChE-stained brain sections using the software package Scion (Scion Corporation, Maryland, USA). For each rat, optical density values obtained from the 3 target brain regions were then normalized to raw striatal optical density values to eliminate the potential influence of different staining intensities across animals (see similar method used by Vuckovich et al. 2004). Raw optical density values from the striatum did not differ between the NBM- and sham-lesioned groups (see Results). The rectangular outlines superimposed on the rat brain schematics depicted in Figure 6 (Paxinos and Watson 2007) delineate the boundaries used for obtaining optical density values in the frontal and parietal cortices and hippocampus.

Results

Statistical Analyses

All statistical analyses were conducted using SPSS Version 15 with an alpha level of 0.05.

Presurgical Performance

Pretraining

Rats required 3 sessions of Light-Water training, 4 sessions of Touch-Light-Water training, 7 sessions of Single-Stimulus training, 16 sessions of Follow-Stimulus training, and a total of 28 sessions of Simultaneous-Discrimination training for criterion performance for all rats to be met.

Visual Search Training

Rats required 12 sessions of Visual Search Set-Size 4 training, 10 sessions of Visual Search Set-Size 6 training, and 18 sessions of Visual Search Set-Size 8 training for criterion performance for all rats to be met. Rats required 12 sessions of Visual Search All Set-Sizes training for stable performance levels to be attained.

Visual Search Task

Figure 3 illustrates the presurgical performance of rats as measured by (A) accuracy and (B) correct latency on the visual search task. Performance has been binned across the 12 sessions of training, and the performance of rats on the 2 different types of Feature Search trials, homogeneous and heterogeneous, was collapsed to yield a single Feature Search score for accuracy and correct latency given that within-subjects analyses of variance (ANOVAs) revealed no significant difference between the accuracy ($F < 3.0$, $\eta^2 = 0.44$) or correct latency ($F < 1.0$, $\eta^2 = 0.04$) of rats on homogeneous and heterogeneous Feature Search trials.

A within-subjects ANOVA was performed using Session, Set-Size (4, 6, and 8 stimuli), and Trial-Type (Feature Search and Conjunctive Search) as within-subjects factors for both accuracy and correct latency. The accuracy ANOVA revealed significant main effects of Set-Size ($F_{1,14,17,10} = 69.26$, $P < 0.001$, $\eta^2 = 0.82$) and Trial-Type ($F_{1,15} = 179.63$, $P < 0.001$, $\eta^2 = 0.92$) as well as a significant Set-Size \times Trial-Type interaction ($F_{1,43,21,39} = 15.13$, $P < 0.001$, $\eta^2 = 0.50$). Within-subjects contrasts revealed that the accuracy of rats was significantly higher on Feature Search than Conjunctive Search trials at all 3 stimulus set-sizes (4 stimuli: $F_{1,15} = 7.44$, $P < 0.05$, $\eta^2 = 0.33$; 6 stimuli: $F_{1,15} = 12.55$, $P < 0.01$, $\eta^2 = 0.46$; and 8 stimuli: $F_{1,15} = 60.28$, $P < 0.001$, $\eta^2 = 0.80$). The correct latency ANOVA revealed a significant main effect of Session ($F_{2,67,40,04} = 1.96$, $P < 0.05$, $\eta^2 = 0.12$), no significant effect of Trial-Type ($F < 3.5$,

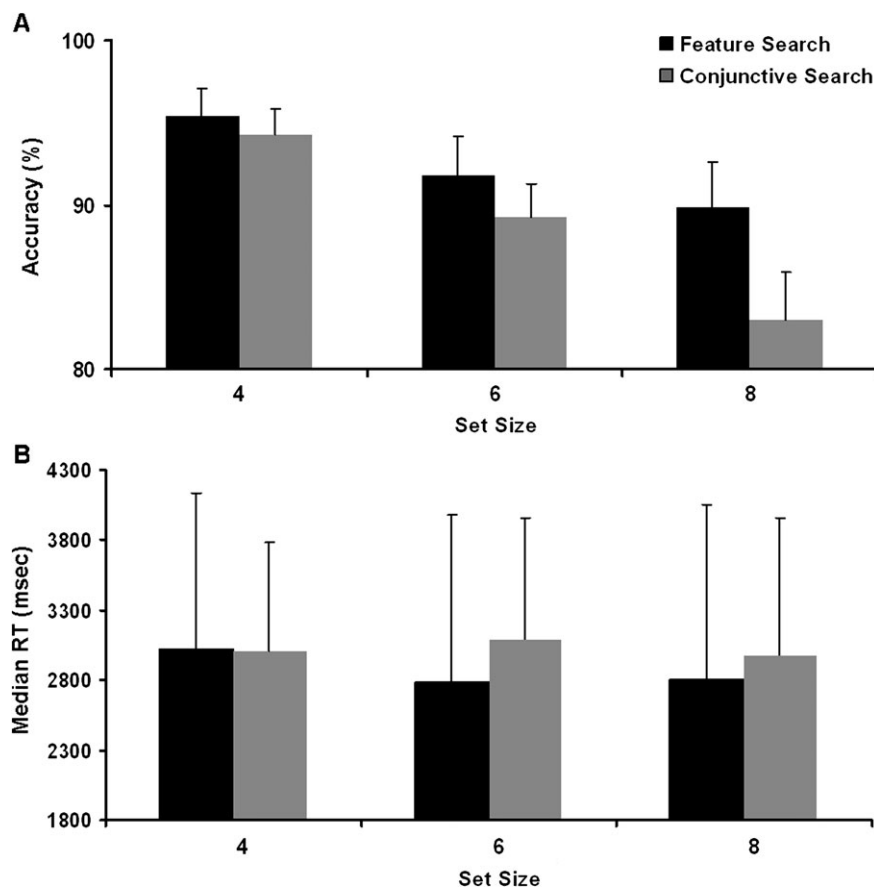


Figure 3. Presurgical performance of rats on the visual search task as measured by (A) accuracy and (B) correct latency. Performance has been binned across the 12 sessions of presurgical training, and the performance of rats on the 2 different types of Feature Search trials, homogeneous and heterogeneous, has been collapsed to yield a single Feature Search score for accuracy and correct latency. + standard error of the mean.

$\eta^2 = 0.18$), and no significant Set-Size \times Trial-Type interaction ($F < 1.0$, $\eta^2 = 0.04$). Two additional ANOVAs were conducted to confirm that the rats assigned to the 2 surgical conditions, sham-lesioned and ACh-NBM-lesioned, did not differ with respect to presurgical visual search accuracy ($F < 1.0$, $\eta^2 = 0.01$) and correct latency ($F < 1.0$, $\eta^2 = 0.02$).

Histological Analyses

Quantitative histological analyses revealed that one ACh-NBM-lesioned rat had a statistically insufficient reduction of ChAT-immunoreactive cells in the NBM as indicated by a ChAT-immunoreactive cell count greater than 2 standard deviations (SDs) above the mean of the ACh-NBM-lesioned group and nonsignificantly different from the mean of the sham-lesioned group. Moreover, the AChE optical density values obtained from the frontal and parietal cortices of this animal were statistically similar to the means of the sham-lesioned group. A second ACh-NBM-lesioned rat was found to have a significant reduction of ChAT-immunoreactive cells in the MS/VDB as indicated by a ChAT-immunoreactive cell count less than 2 SDs below the MS/VDB means of both the ACh-NBM-lesioned and the sham-lesioned groups. Furthermore, the AChE optical density value obtained from the hippocampus of this animal was significantly lower than the means of both the ACh-NBM-lesioned and the sham-lesioned groups. Accordingly, behavioral and histological data from these 2 rats were removed from

presurgical and postsurgical statistical analyses. The sham-lesioned sample size thus remained at 8 rats, while the ACh-NBM-lesioned sample size was reduced from 10 to 8 rats.

Cell Counting

There was significant depletion of ChAT-immunoreactive cells in the NBM of ACh-NBM-lesioned rats relative to sham-lesioned rats (Fig. 4), while an equivalent number of ChAT-immunoreactive cells was observed in the neighboring MS/VDB nuclei of both groups of rats (Fig. 5). Independent *t*-tests confirmed that there were significantly fewer ChAT-immunoreactive cells in the NBM of ACh-NBM-lesioned compared with sham-lesioned rats ($t_{12} = 10.5$, $P < 0.001$; $M_{\text{Sham}} = 299.29$, $SD = 45.04$; $M_{\text{NBM}} = 147.71$, $SD = 20.33$). However, there was no significant difference between the 2 groups of rats in the number of ChAT-immunoreactive cells in the MS/VDB ($t < 2.5$; $M_{\text{Sham}} = 458.71$, $SD = 36.10$; $M_{\text{NBM}} = 448.43$, $SD = 21.55$). An equivalent number of parvalbumin-immunoreactive cells was observed in the NBM (Fig. 4) and MS/VDB (Fig. 5) of both sham- and ACh-NBM-lesioned rats. Independent *t*-tests confirmed that there were no significant differences, $t < 2.0$, between the 2 groups of rats in the number of parvalbumin-positive cells in the NBM ($M_{\text{Sham}} = 114.00$, $SD = 13.17$; $M_{\text{NBM}} = 124.71$, $SD = 8.46$) and MS/VDB ($M_{\text{Sham}} = 373.00$, $SD = 45.60$; $M_{\text{NBM}} = 397.43$, $SD = 48.01$). It should be noted that the neural tissue of 1 sham-lesioned and 1 ACh-NBM-lesioned rat was too fragile due to insufficient paraformaldehyde tissue penetration to

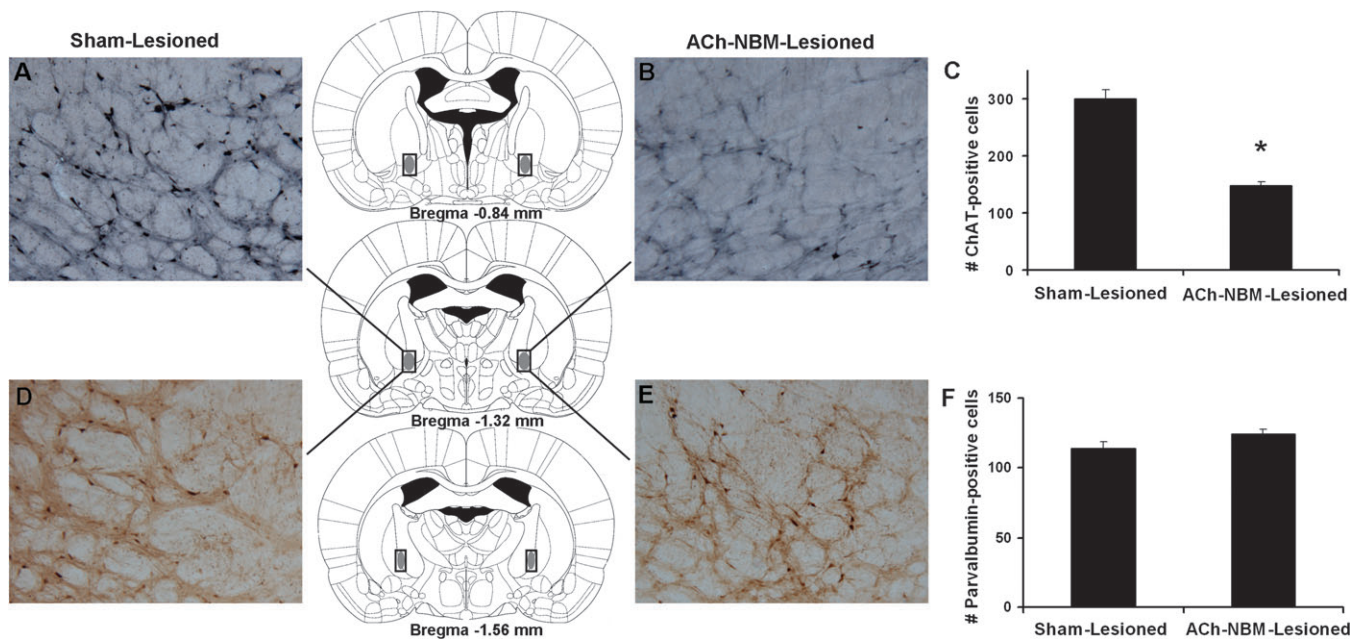


Figure 4. ChAT and parvalbumin immunohistochemistry of the NBM. Displayed are a typical sham-lesioned rat on the left and a typical ACh-NBM-lesioned rat on the right (magnification 10 \times). The rectangular outlines superimposed on the rat brain coronal schematics delineate the NBM cell-counting frames, and the solid gray fill illustrates the typical extent of ChAT-immunoreactive cell loss. The number of ChAT-immunoreactive cells in the NBM (A,B) was significantly reduced in the ACh-NBM-lesioned rats (C). There was no significant difference in the number of parvalbumin-immunoreactive cells between the 2 groups (D,E) in the NBM (F). + standard error of the mean; * $P < 0.05$. Rat brain schematics adapted from Paxinos and Watson (2007).

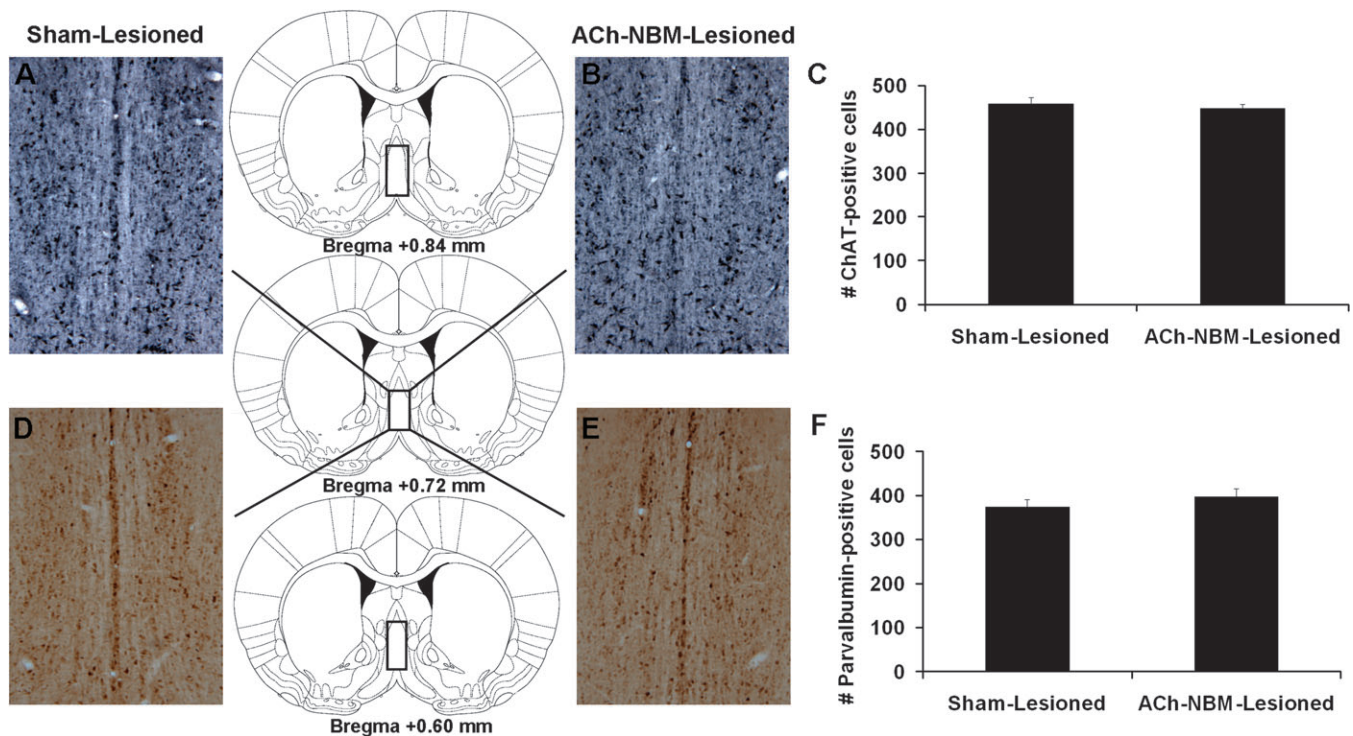


Figure 5. ChAT and parvalbumin immunohistochemistry of the MS/VDB. Displayed are a typical sham-lesioned rat on the left and a typical ACh-NBM-lesioned rat on the right (magnification 10 \times). The rectangular outlines superimposed on the rat brain coronal schematics delineate the MS/VDB cell-counting frames. There was no significant difference in the number of ChAT-immunoreactive cells between the 2 groups (A,B) in the MS/VDB (C), and there was no significant difference in the number of parvalbumin-immunoreactive cells between the 2 groups (D,E) in the MS/VDB (F). + standard error of the mean. Rat brain schematics adapted from Paxinos and Watson (2007).

endure immunohistochemical analyses. Thus, for these 2 animals, histological analyses and quantification were based solely on AChE densitometry.

AChE Densitometry

AChE staining revealed a loss of cholinergic fibers in the frontal and parietal cortices, but not the hippocampus of ACh-NBM-

lesioned rats relative to sham-lesioned rats (Fig. 6). This was confirmed by independent *t*-tests comparing the optical density values (normalized to raw striatal optical density values) from the 3 target brain regions between the 2 groups of rats. The raw optical density values from the striatum (used for normalization) did not differ between the 2 groups ($t < 1.0$; $M_{\text{Sham}} = 247.33$, $SD = 12.31$; $M_{\text{NBM}} = 251.38$, $SD = 1.32$). There was significantly less AChE reactivity as measured by optical density in the frontal ($t_{14} = 4.62$, $P < 0.001$; $M_{\text{Sham}} = 0.50$, $SD = 0.07$; $M_{\text{NBM}} = 0.37$, $SD = 0.03$) and parietal ($t_{14} = 5.34$, $P < 0.001$; $M_{\text{Sham}} = 0.48$, $SD = 0.05$; $M_{\text{NBM}} = 0.35$, $SD = 0.04$) cortices of ACh-NBM-lesioned compared with sham-lesioned rats. However, there was no significant difference between the 2 groups of rats in AChE reactivity in the hippocampus as measured by optical density ($t < 2.0$; $M_{\text{Sham}} = 0.52$, $SD = 0.04$; $M_{\text{NBM}} = 0.48$, $SD = 0.04$).

Postsurgical Performance

The postsurgical performance of sham-lesioned rats on the visual search task was comparable to their presurgical performance: 2 within-subjects ANOVAs revealed no significant difference between the presurgical and postsurgical visual search accuracy ($F < 1.0$, $\eta^2 = 0.02$) and correct latency ($F < 2.0$, $\eta^2 = 0.12$) of sham-lesioned rats. Furthermore, consistent with the presurgical analyses, a within-subjects

ANOVA using the postsurgical accuracy data (12 sessions) from sham-lesioned rats revealed significant main effects of Set-Size ($F_{2,14} = 55.05$, $P < 0.001$, $\eta^2 = 0.89$) and Trial-Type ($F_{1,7} = 100.22$, $P < 0.001$, $\eta^2 = 0.94$) as well as a significant Set-Size \times Trial-Type interaction ($F_{2,14} = 29.54$, $P < 0.001$, $\eta^2 = 0.81$). Likewise, a within-subjects ANOVA using the postsurgical correct latency data (12 sessions) from sham-lesioned rats revealed no significant effects of Set-Size ($F < 2.0$, $\eta^2 = 0.20$) and Trial-Type ($F < 1.0$, $\eta^2 = 0.12$) and no significant Set-Size \times Trial-Type interaction ($F < 2.0$, $\eta^2 = 0.24$).

To determine the impact of reduced cortical cholinergic afferentation on visual search performance for both the postsurgical accuracy and the correct latency data, 2 mixed ANOVAs were performed, 1 for Feature Search and 1 for Conjunctive Search trials, using Session and Set-Size (4, 6, and 8 stimuli) as within-subjects factors and Lesion (sham-lesioned or ACh-NBM-lesioned) as a between-subjects factor. The performance of rats on the 2 different types of Feature Search trials, homogeneous and heterogeneous, was collapsed to yield a single Feature Search score for accuracy and correct latency given that within-subjects ANOVAs revealed no significant difference between the homogeneous and the heterogeneous Feature Search trial accuracy or correct latency of ACh-NBM-lesioned (accuracy: $F < 3.0$, $\eta^2 = 0.62$; correct latency: $F < 1.0$, $\eta^2 = 0.01$) and sham-lesioned (accuracy: $F < 3.0$, $\eta^2 = 0.04$; correct latency: $F < 1.0$, $\eta^2 = 0.05$) rats. Furthermore,

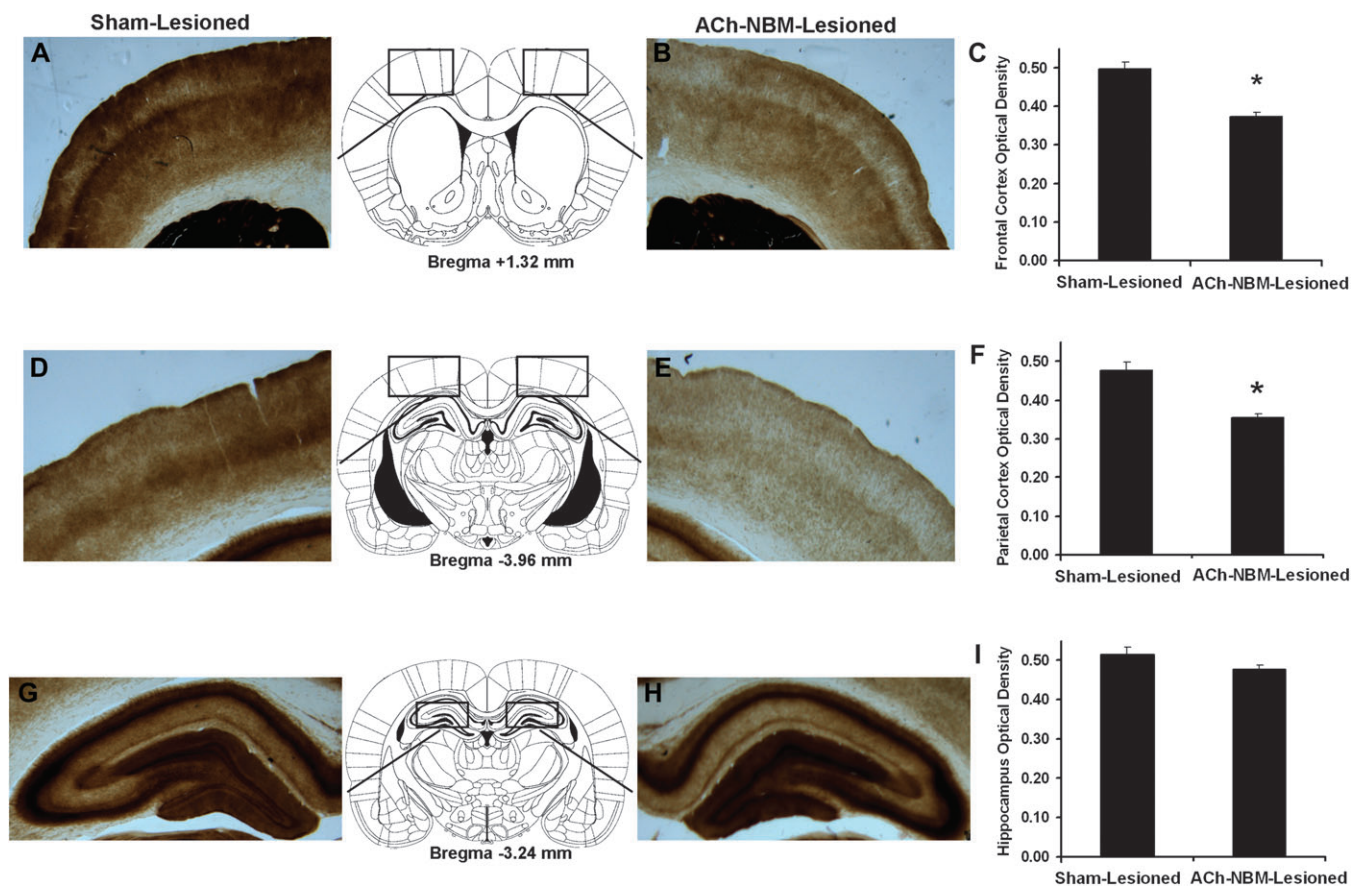


Figure 6. AChE histochemistry. Displayed are a typical sham-lesioned rat on the left and a typical ACh-NBM-lesioned rat on the right (magnification 1.25 \times). The rectangular outlines superimposed on the rat brain coronal schematics delineate the boundaries used for obtaining optical density values (normalized to raw striatal optical density values) in the frontal and parietal cortices and hippocampus. The degree of AChE-positive staining as measured by optical density in the frontal (A,B) and parietal cortices (D,E) was significantly reduced in the ACh-NBM-lesioned rats (C,F). There was no significant difference in the amount of AChE-positive staining in the hippocampus as measured by optical density (G,H) between the NBM- and sham-lesioned groups (I). + standard error of the mean; * $P < 0.05$. Rat brain schematics adapted from Paxinos and Watson (2007).

ACh-NBM-lesioned rats performed comparably to that of sham-lesioned rats on heterogeneous Feature Search trials as measured by accuracy ($F < 1.0$, $\eta^2 = 0.06$) and correct latency ($F < 2.0$, $\eta^2 = 0.11$).

For the postsurgical accuracy data (12 sessions), the Feature Search ANOVA revealed significant main effects of Session ($F_{11,154} = 3.68$, $P < 0.001$, $\eta^2 = 0.21$) and Set-Size ($F_{2,28} = 31.71$, $P < 0.001$, $\eta^2 = 0.69$), and no significant effect of Lesion ($F < 1.0$, $\eta^2 = 0.04$). The Conjunctive Search accuracy ANOVA revealed significant main effects of Session ($F_{11,154} = 3.46$, $P < 0.001$, $\eta^2 = 0.20$) and Set-Size ($F_{1,33,18,60} = 74.76$, $P < 0.001$, $\eta^2 = 0.84$), and no significant effect of Lesion ($F < 1.5$, $\eta^2 = 0.09$). For the postsurgical correct latency data (12 sessions), the Feature Search ANOVA revealed a significant main effect of Session ($F_{3,14,44,04} = 1.96$, $P < 0.05$, $\eta^2 = 0.12$) and no significant effect of Lesion ($F < 1.5$, $\eta^2 = 0.10$). The Conjunctive Search correct latency ANOVA revealed significant main effects of Set-Size ($F_{2,26} = 3.91$, $P < 0.05$, $\eta^2 = 0.23$) and Lesion ($F_{1,13} = 3.89$, $P = 0.05$, $\eta^2 = 0.23$).

Between-subjects contrasts revealed significant differences between the conjunctive search correct latency of sham-lesioned and ACh-NBM-lesioned rats at set sizes of 6 stimuli ($F_{1,13} = 5.12$, $P < 0.05$, $\eta^2 = 0.28$; $M_{\text{Sham-Lesioned}} = 2715.89$, $SD = 623.12$; $M_{\text{ACh-NBM-Lesioned}} = 3197.82$, $SD = 615.11$) and 8 stimuli ($F_{1,13} = 2.70$, $P = 0.05$, $\eta^2 = 0.18$; $M_{\text{Sham-Lesioned}} = 2759.09$,

$SD = 548.20$; $M_{\text{ACh-NBM-Lesioned}} = 3131.17$, $SD = 581.93$), but not 4 stimuli ($F < 1.0$, $\eta^2 = 0.05$; $M_{\text{Sham-Lesioned}} = 3010.54$, $SD = 714.48$; $M_{\text{ACh-NBM-Lesioned}} = 3290.83$, $SD = 645.31$). To illustrate the selective impact of reduced cortical cholinergic afferentation on conjunctive search correct latency at the largest stimulus set sizes, Figure 7 depicts the cost of ACh-NBM lesions on visual search accuracy and correct latency during Feature and Conjunctive Search trials at all 3 stimulus set sizes. The cost of ACh-NBM lesions was computed by calculating a difference (Δ) score (mean accuracy or correct latency of sham-lesioned rats – mean accuracy or correct latency of ACh-NBM-lesioned rats) for both Feature and Conjunctive Search trials using data averaged across all 12 postsurgical testing sessions.

Discussion

We have previously shown that systemic muscarinic cholinergic receptor blockade with scopolamine (Botly and De Rosa 2007, 2008) and destruction of cholinergic neurons in the NBM of the basal forebrain (Botly and De Rosa 2009) impaired the ability of rats to acquire a digging-based feature binding task. This task required the binding of olfactory and texture stimulus features, but the binding was associative in nature, and the competition from distractor stimuli was indirect such that

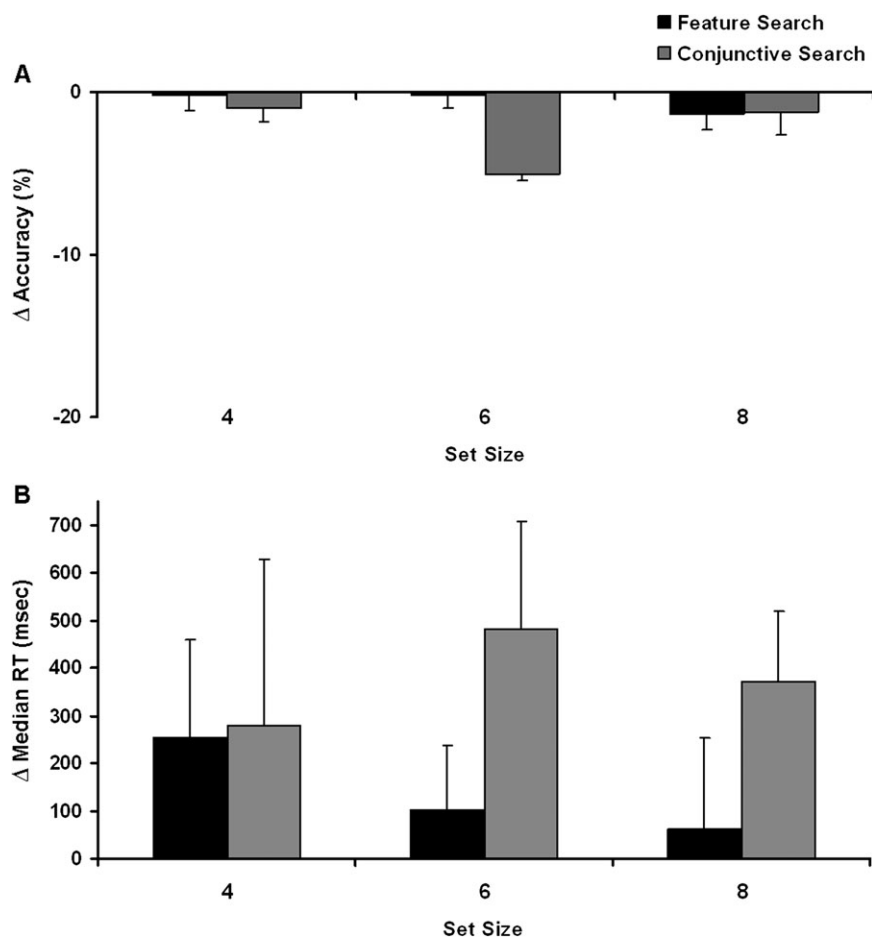


Figure 7. Performance costs of cholinergic-selective NBM lesions on accuracy (A) and correct latency (B) during Feature and Conjunctive Search trials of the visual search task. The cost of ACh-NBM lesions was computed by calculating a difference (Δ) score (mean accuracy or correct latency of sham-lesioned rats – mean accuracy or correct latency of ACh-NBM-lesioned rats) for both Feature and Conjunctive Search trials at all 3 stimulus set sizes using data averaged across all 12 postsurgical testing sessions. \pm standard error of the mean.

visuospatial attention was not specifically taxed. Thus, the question of whether one function of cortical cholinergic contributions to visuospatial attention is its importance to feature binding remained to be tested. In the present study, we designed a rodent analog of the standard test of human visuospatial attention and feature binding known as visual search. We predicted that a reduction of cortical cholinergic afferentation would disrupt visuospatial attention and result in less efficient visual search.

Extensive use of the visual search task with humans has revealed that latency to locate the target stimulus, relative to accuracy, is a more sensitive measure of attentional dysfunction (Treisman and Gelade 1980). Moreover, behavioral and functional neuroimaging research and work with clinical populations characterized by cholinergic disruption and attentional impairments, such as Alzheimer's disease (AD), suggest that the increased attentional demands associated with locating a conjunction of features amongst an array of numerous distractors is the cause of the increased latency needed to locate the target stimulus on these trials (Corbetta et al. 1995; Treisman 1998; Foster et al. 1999; Donner et al. 2002; Tales et al. 2002; Esterman et al. 2007). Given this demonstrated sensitivity of the visual search latency measure to cholinergic and attentional disruption, we expected that our manipulation of the cortical cholinergic system would solely influence latency in rats.

As predicted, ACh-NBM-lesioned rats took significantly longer than sham-lesioned rats to locate the target stimulus on Conjunctive Search, but not Feature Search trials. Furthermore, this significant lesion-induced increase in correct latency was found only during Conjunctive Search trials with the larger stimulus set sizes of 6 or 8; specifically, ACh-NBM-lesioned rats took approximately 400–500 ms longer than sham-lesioned rats to locate the target stimulus on Conjunctive Search trials at these stimulus set sizes. This is consistent with the notion that as the number of distractors increased on Conjunctive Search trials, a more extensive serial search was necessary to locate the target that required visuospatial attentional resources that were not available to ACh-NBM-lesioned rats.

It is important to note that the only difference between Conjunctive and Feature Search trials was whether feature binding was required, which we manipulated by presenting distractor stimuli that differed from the target on either 1 (Feature Search trials) or 2 (Conjunctive Search trials) feature dimensions. We took care to control for the heterogeneity of the conjunctive search displays by including Feature Search trials in which the distractor stimuli presented were not identical (Fig. 2). Critically, ACh-NBM-lesioned rats performed no differently than sham-lesioned control rats on these heterogeneous Feature Search trials indicating that it was not simply the heterogeneity of the Conjunctive Search trial displays that contributed to the lesion-induced increase in conjunctive search latency. Moreover, if lower level cognitive processes such as discrimination ability were impacted by the destruction of cholinergic NBM neurons, then, we would have expected to find that ACh-NBM-lesioned rats were also slower at locating the target stimulus on Feature Search trials because the target stimulus, distractor stimuli, and number of distractor stimuli presented were equivalent across Feature Search and Conjunctive Search trials. We would also have expected to find that ACh-NBM-lesioned rats were less accurate than sham-lesioned controls at correctly locating the target stimulus if

discrimination ability was impaired, which was not the case. Such intact accuracy, but selectively increased latency at correctly locating the target stimulus on Conjunctive Search trials following surgery, suggests that ACh-NBM-lesioned rats were fully capable of correctly performing the visual search task but were less efficient at conjunctive search than sham-lesioned controls.

These findings implicating cholinergic input to the cortex in efficient visual search performance are consistent with non-human animal electrophysiological (Gill et al. 2000; Broussard et al. 2006; Herrero et al. 2008; Broussard et al. 2009) and microdialysis (Himmelheber et al. 1997; Passetti et al. 2000; Himmelheber et al. 2001; Arnold et al. 2002; Kozak et al. 2006; Parikh et al. 2007; Parikh and Sarter 2008) research implicating cortical ACh in attention. For instance, in vivo microdialysis work using freely behaving rats has revealed that cholinergic neurotransmission in frontoparietal cortical regions is important for attentional processing. Increasing the attentional demands of a visual sustained attention task by introducing a distracting stimulus resulted in a substantial increase in ACh efflux in the frontal and parietal cortices of rats (Himmelheber et al. 2000; Arnold et al. 2002), suggesting that high levels of ACh in these cortical regions are needed to support the increased attentional demands of the task.

A classic visual search performance pattern well documented by the human cognitive literature is a significant increase in the latency to locate the target stimulus as the number of distractors increases on Conjunctive, but not Feature Search trials. The positive slope of the conjunctive search latency curve is considered to be due to the occurrence of a serial-like search for the target. However, analyses conducted on the presurgical correct latency data did not find that rats took significantly longer to locate the target stimulus on Conjunctive versus Feature Search trials. Given that rats received a significant amount of visual search training prior to surgery it is possible that such overtraining may have significantly attenuated any trial-type differences in correct latency, which has been shown to occur in pigeons (Vreven and Blough 1998). However, a closer examination of our training data failed to reveal any initial significant differences between Feature and Conjunctive Search correct latency. It should be noted that in order to keep the size of the stimuli large enough for rats to accurately discriminate, our largest stimulus set size comprised only 8 stimuli, which is much smaller than the set sizes of approximately 20 stimuli typically employed with human participants (Nobre et al. 2003). Thus, our maximum stimulus set size may not have been large enough to yield significant trial-type interactions with latency. Interestingly, like humans, pigeons also yield latency-based feature–conjunctive differences when large stimulus set sizes of 20 or more stimuli are used (Blough 1989). This suggests that rats may also yield such latency-based feature–conjunctive differences if larger stimulus set sizes could be employed. Importantly, while the relatively small stimulus set sizes used in the present study may not have been sufficient to induce the typical latency-based visual search performance pattern prior to surgery, disruption of the cortical cholinergic system allowed for a latency-based performance pattern to emerge following surgery as ACh-NBM-lesioned rats took significantly longer than sham-lesioned rats to locate the target stimulus on Conjunctive Search, but not Feature Search trials.

Analyses conducted on the presurgical accuracy data revealed that rats were significantly more accurate at locating the target stimulus on Feature Search than Conjunctive Search trials. Importantly, stimulus set size was found to significantly interact with trial type such that as the number of distractors increased, rats' accuracy at locating the target stimulus decreased on Conjunctive Search trials, while remaining relatively more stable on Feature Search trials. This accuracy-based pattern of performance demonstrated by rats resembles the classic latency-based performance pattern typically observed in human participants and suggests that the feature binding requirement of Conjunctive Search trials made it more challenging for rats to accurately locate the target stimulus, especially as the number of distractors increased. This is consistent with findings from the human cognitive literature demonstrating that feature binding errors, like illusory conjunctions, are more likely to occur under conditions of high attentional load, such as when conjunctive search with large stimulus set sizes is required (Treisman and Schmidt 1982; Cinel et al. 2002). Furthermore, in a cross-species study in which pigeons and human participants performed visual search using the same search displays, pigeons primarily showed feature-conjunctive differences in accuracy, while human participants showed feature-conjunctive differences in latency (Cook 1992).

Overall, this study provides further support for the cholinergic hypothesis of feature binding using the well-established visuospatial attention task from the human cognitive literature known as visual search. Specifically, we have demonstrated that cortical ACh's important functional role in visuospatial attention extends to feature binding and we have revealed that rats are capable of successfully performing a visual search task, which to our knowledge has not been done before. The use of touch screen-based cognitive assessment is on the rise in the behavioral neuroscience literature (Markham et al. 1996; Bussey et al. 2001; Cook et al. 2004; Bussey et al. 2008; McTighe et al. 2009; Talpos et al. 2009), and this rodent visual search paradigm could be adapted for use as an additional cognitive paradigm for testing rodent models of neurodegenerative diseases associated with cholinergic function, such as AD.

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References

Arnold HM, Burk JA, Hodgson EM, Sarter M, Bruno JP. 2002. Differential cortical acetylcholine release in rats performing a sustained attention task versus behavioral control tasks that do not explicitly tax attention. *Neuroscience*. 114:451-460.

Balan PF, Oristaglio J, Schneider DM, Gottlieb J. 2008. Neuronal correlates of the set-size effect in monkey lateral intraparietal area. *PLoS Biol*. 6:e158.

Blough PM. 1984. Visual search in pigeons: effects of memory set size and display variables. *Percept Psychophys*. 35:344-352.

Blough DS. 1989. Odd-item search in pigeons: display size and transfer effects. *J Exp Psychol Anim Behav Process*. 15:14-22.

Botly LCP, De Rosa E. 2007. Cholinergic influences on feature binding. *Behav Neurosci*. 121:264-276.

Botly LCP, De Rosa E. 2008. A cross-species investigation of acetylcholine, attention, and feature binding. *Psychol Sci*. 19:1185-1193.

Botly LCP, De Rosa E. 2009. Cholinergic deafferentation of the neocortex using 192 IgG-saporin impairs feature binding in rats. *J Neurosci*. 29:4120-4130.

Broussard JJ, Karelina K, Sarter M, Givens B. 2009. Cholinergic optimization of cue-evoked parietal activity during challenged attentional performance. *Eur J Neurosci*. 29:1711-1722.

Broussard JJ, Sarter M, Givens B. 2006. Neuronal correlates of signal detection in the posterior parietal cortex of rats performing a sustained attention task. *Neuroscience*. 143:407-417.

Bussey TJ, Padain TL, Skillings EA, Winters BD, Morton AJ, Saksida LM. 2008. The touchscreen cognitive testing method for rodents: how to get the best out of your rat. *Learn Mem*. 15:516-523.

Bussey TJ, Saksida LM, Rothblat LA. 2001. Discrimination of computer-graphic stimuli by mice: a method for the behavioral characterization of transgenic and gene-knockout models. *Behav Neurosci*. 115:957-960.

Cinel C, Humphreys GW, Poli R. 2002. Cross-modal illusory conjunctions between vision and touch. *J Exp Psychol Hum Percept Perform*. 28:1243-1266.

Cook RG. 1992. Dimensional organization and texture discrimination in pigeons. *J Exp Psychol Anim Behav Process*. 18:354-363.

Cook RG, Geller AI, Zhang G, Gowda R. 2004. Touchscreen-enhanced visual learning in rats. *Behav Res Methods Instrum Comput*. 36:101-106.

Corbetta M, Shulman GL, Miezin FM, Petersen SE. 1995. Superior parietal cortex activation during spatial attention shifts and visual feature conjunction. *Science*. 270:802-805.

Donner TH, Kettermann A, Diesch E, Ostendorf F, Villringer A, Brandt SA. 2002. Visual feature and conjunction searches of equal difficulty engage only partially overlapping frontoparietal networks. *Neuroimage*. 15:16-25.

Esterman M, Verstynen T, Robertson LC. 2007. Attenuating illusory binding with TMS of the right parietal cortex. *Neuroimage*. 35:1247-1255.

Foster JK, Behrmann M, Stuss DT. 1999. Visual attention deficits in Alzheimer's disease: simple versus conjoined feature search. *Neuropsychology*. 13:223-245.

Gill TM, Sarter M, Givens B. 2000. Sustained visual attention performance-associated prefrontal neuronal activity: evidence for cholinergic modulation. *J Neurosci*. 20:4745-4757.

Harati H, Barbelivien A, Cosquer B, Majchrzak M, Cassel JC. 2008. Selective cholinergic lesions in the rat nucleus basalis magnocellularis with limited damage in the medial septum specifically alter attention performance in the five-choice serial reaction time task. *Neuroscience*. 153:72-83.

Herrero JL, Roberts MJ, Delicato LS, Gieselmann MA, Dayan P, Thiele A. 2008. Acetylcholine contributes through muscarinic receptors to attentional modulation in V1. *Nature*. 454:1110-1114.

Himmelheber AM, Sarter M, Bruno JP. 1997. Operant performance and cortical acetylcholine release: role of response rate, reward density, and non-contingent stimuli. *Brain Res Cogn Brain Res*. 6:23-36.

Himmelheber AM, Sarter M, Bruno JP. 2000. Increases in cortical acetylcholine release during sustained attention performance in rats. *Brain Res Cogn Brain Res*. 9:313-325.

Himmelheber AM, Sarter M, Bruno JP. 2001. The effects of manipulations of attentional demand on cortical acetylcholine release. *Brain Res Cogn Brain Res*. 12:353-370.

Kozak R, Bruno JP, Sarter M. 2006. Augmented prefrontal acetylcholine release during challenged attentional performance. *Cereb Cortex*. 16(1):9-17.

- Lehmann O, Grottick AJ, Cassel J-C, Higgins GA. 2003. A double dissociation between serial reaction time and radial maze performance in rats subjected to 192 IgG-saporin lesions of the nucleus basalis and/or the septal region. *Eur J Neurosci.* 18:651-666.
- Markham MR, Butt AE, Dougher MJ. 1996. A computer touch-screen apparatus for training visual discriminations in rats. *J Exp Anal Behav.* 65:173-182.
- McGaughy J, Dalley JW, Morrison CH, Everitt BJ, Robbins TW. 2002. Selective behavioral and neurochemical effects of cholinergic lesions produced by intrabasalis infusions of 192 IgG-Saporin on attentional performance in a five-choice serial reaction time task. *J Neurosci.* 22:1905-1913.
- McTighe SM, Mar AC, Romberg C, Bussey TJ, Saksida LM. 2009. A new touchscreen test of pattern separation: effect of hippocampal lesions. *Neuroreport.* 20:881-885.
- Nobre AC, Coull JT, Walsh V, Frith CD. 2003. Brain activations during visual search: contributions of search efficiency versus feature binding. *Neuroimage.* 18:91-103.
- Nothdurft HC, Pigarev IN, Kastner S. 2009. Overt and covert visual search in primates: reaction times and gaze shift strategies. *J Integr Neurosci.* 8:137-174.
- Parikh V, Kozak R, Martinez V, Sarter M. 2007. Prefrontal acetylcholine release controls cue detection on multiple timescales. *Neuron.* 56:141-154.
- Parikh V, Sarter M. 2008. Cholinergic mediation of attention: contributions of phasic and tonic increases in prefrontal cholinergic activity. *Ann N Y Acad Sci.* 1129:225-235.
- Passetti F, Dalley JW, O'Connell MT, Everitt BJ, Robbins TW. 2000. Increased acetylcholine release in the rat medial prefrontal cortex during performance of a visual attentional task. *Eur J Neurosci.* 12:3051-3058.
- Paxinos G, Watson C. 1998. *The rat brain in stereotaxic coordinates.* 4th ed. San Diego (CA): Academic Press.
- Paxinos G, Watson C. 2007. *The rat brain in stereotaxic coordinates.* 6th ed. Burlington (MA): Academic Press.
- Sarter M, Bruno JP, Givens B. 2003. Attentional functions of cortical cholinergic inputs: what does it mean for learning and memory? *Neurobiol Learn Mem.* 80:245-256.
- Tales A, Butler SR, Fossey J, Gilchrist ID, Jones RW, Troscianko T. 2002. Visual search in Alzheimer's disease: a deficiency in processing conjunctions of features. *Neuropsychologia.* 40:1849-1857.
- Talpos JC, Winters BD, Dias R, Saksida LM, Bussey TJ. 2009. A novel touchscreen-automated paired-associate learning (PAL) task sensitive to pharmacological manipulation of the hippocampus: a translational rodent model of cognitive impairments in neurodegenerative disease. *Psychopharmacology (Berl).* 205:157-168.
- Treisman A. 1998. Feature binding, attention and object perception. *Philos Trans R Soc Lond B Biol Sci.* 353:1295-1306.
- Treisman A, Gelade G. 1980. A feature-integration theory of attention. *Cogn Psychol.* 12:97-136.
- Treisman A, Schmidt H. 1982. Illusory conjunctions in the perception of objects. *Cogn Psychol.* 14:107-141.
- Vreven D, Blough PM. 1998. Searching for one or many targets: effects of extended experience on the runs advantage. *J Exp Psychol Anim Behav Process.* 24:98-105.
- Vuckovich JA, Semel ME, Baxter MG. 2004. Extensive lesions of cholinergic basal forebrain neurons do not impair spatial working memory. *Learn Mem.* 11:87-94.