The nucleus basalis magnocellularis contributes to feature binding in the rat

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\textbf{ABSTRACT}

The binding problem refers to the fundamental challenge of the central nervous system to integrate sensory information registered by multiple brain regions to form a unified neural representation of a stimulus. Although the human cognitive literature has yielded substantial insights into the attention-dependent nature and general cortical networks involved in feature binding, the specific downstream neuroanatomical contributions to feature binding remain unknown. We hypothesized that the nucleus basalis magnocellularis (NBM) of the basal forebrain would be critical for feature binding given the NBM’s widespread neuromodulatory projections to regions of the neocortex important for attentional processing, such as the frontal and parietal cortices. Accordingly, we tested the ability of rats with bilateral excitotoxic (quisqualic acid) lesions of the NBM to acquire a crossmodal Feature-Conjunction (FC) task that required feature binding and a Feature-Singleton (FS) task that did not require feature binding. Additionally, rats retrieved a FC stimulus set they had acquired prior to surgery. Relative to sham-lesioned controls, NBM-lesioned rats were significantly impaired at acquiring and retrieving the FC task, while their ability to acquire the FS task remained intact. These findings provide insight into the functional role of the NBM and establish the importance of this basal forebrain structure to the fundamental cognitive process of feature binding.

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1. Introduction

We are capable of perceiving objects as a unified whole even though the mammalian brain is organized in a modular fashion; that is, distinct neural regions are primarily responsible for detecting and processing the different features of an object, such as its shape or colour. The unknown mechanism by which a unified neural representation of a stimulus is formed is referred to as feature binding [1,2]. Feature binding tasks have been widely employed in the human cognitive literature to investigate the cognitive processes and cortical networks involved in feature binding and such tasks typically involve the presentation of two different types of stimuli, Feature-Singleton (FS) and Feature-Conjunction (FC). While FS stimuli only require the processing of single features (e.g. find a yellow apple in a basket of green apples and pears), FC stimuli require the binding of multiple features (e.g. find a yellow apple within a basket of green apples and green and yellow pears). Patients with attentional impairments are impaired at FC, but not FS tasks [3–7], and functional magnetic resonance imaging (fMRI) studies have implicated frontoparietal cortical networks in feature binding [8–14].

While the human cognitive literature has yielded substantial insights into the cognitive mechanisms and broad neocortical systems involved in feature binding, the specific downstream neuroanatomical contributions to feature binding remain unknown. The basal forebrain (BF) projection system has a complex anatomical and neurochemical composition and is customarily defined by magnocellular cholinergic neurons. The BF projection system is topographically organized, where, in simple terms, the anterior portion (medial septum/vertical limb of diagonal band of Broca; MS/VDB) projects primarily to the hippocampus and related structures and the posterior portion (nucleus basalis magnocellularis; NBM) projects to the neocortex. Hence, we hypothesized that the NBM would be critical for feature binding given its demonstrated importance to a variety of forms of attention, including sustained, selective, and divided attention [15–20], and its widespread neuromodulatory input to regions of the neocortex important for attentional processing, including the frontal and parietal cortices [21].

We set out to investigate whether the NBM was an important contributor to feature binding using a rodent feature binding task that employed the same forced-choice digging paradigm as that used in our previous systemic pharmacology work [22]. To determine whether the NBM had a functional role in feature binding, we tested the ability of rats with bilateral quisqualic acid lesions of the NBM to acquire crossmodal FC and FS stimuli as well as retrieve a pre-surgery FC stimulus set. In our feature binding task, rats are simultaneously presented with two digging bowls on every trial: a crossmodal odor–texture bowl covered with a texture and scented with an odor, and a...
blank bowl with no odor or texture components. Only one of the diggings bowls is baited with a food reward on any given trial. In the FC task, rats must bind odor and texture features for correct bowl selection, while in the FS task they can rely on a single feature (odor or texture).

2. Methods

2.1. Subjects

Subjects were 30 experimentally naïve male Long–Evans rats (Charles River, Montreal, Quebec) that weighed 216–240 g at the start of the experiment. Rats were housed individually in 45 cm long×25 cm wide plastic tub cages and maintained on a reversed 12 h light–12 h dark cycle (lights off at 8 am) with testing occurring during the dark phase (between 10 am and 4 pm). Rats were maintained at 90% of ad libitum free-feeding weight for the duration of the experiment. This study was approved by the University of Toronto’s Institutional Animal Care Committee.

2.2. Apparatus

The training environment was a black Plexiglas chamber 30.5 cm high×76.2 cm long×45.7 cm wide. A black slide-in-door of the same material separated the chamber into two compartments (Fig. 1). The door was positioned 25.4 cm from the back wall of the chamber creating a “start box” (25.4 cm long×45.7 cm wide) and a “testing arena” (50.8 cm long×45.7 cm wide). The experimental room was illuminated by a single 60-W light bulb. The digging apparatus was positioned on a table next to a computer equipped with speakers that emitted white noise and ambient voices to mask any extraneous noises.

2.3. Odor-texture digging bowls

All digging bowls (8 cm deep×4 cm diameter) were painted matte black and attached to heavy 10-cm-square 4-mm-thick black metal bases. The blank bowl had no additional sensory attributes. In contrast, the outside surface, rim, and metal base of each of the odor-texture bowls was covered by a textured cloth using silicon glue, which allowed for the easy removal of the textures. To minimize the tendency for rats to use visual cues to discriminate the textures, all textures were various shades of brown. Glued to the bottom interior surface of each odor-texture bowl was a small metal cap (8 cm in diameter×1 cm high) containing cotton gauze. Each cap contained 32 small holes 3 mm in diameter. At the beginning of each training day and after every four rats completed their sessions, the gauze was re-injected with 0.1 ml of the appropriate scented undiluted aromatherapy oil (Aveda®, Blain, MN; The Body Shop®, Wake Forest, NC). As an additional precaution, prior to beginning each rat’s session, every odor-texture digging bowl was smelled by the experimenter to ensure that each odor was detectible to the human nose and thereby detectible to the rats given their more sensitive olfactory system. Although such an occurrence was rare, if an odor was not detected by the experimenter, additional aromatherapy oil (0.1 ml at a time) was injected until the odor became detectible. Before bowls were reused for novel stimuli, they were thoroughly soaked in rubbing alcohol for three days and spray painted to remove any lingering odor. Table 1 lists the odorants and textures from which the experimental stimuli were created.

2.4. Forced-choice digging paradigm

Fig. 1 illustrates the two different trial types (A), Target and Distractor, of the forced-choice digging tasks. On Target trials, the rewarded (+) stimulus was the odor-texture bowl, while on Distractor trials, the rewarded stimulus was the blank bowl. B: Illustration of a typical session. On every trial, rats were simultaneously presented with two digging bowls: an odor-texture bowl and the blank bowl. Half of the trials were Target (T) trials and the remaining half were Distractor (D) trials presented in a pseudorandom order. Rats had to use the crossmodal features of the presented odor-texture bowl to determine the correct bowl choice.

Table 1

<table>
<thead>
<tr>
<th>Aromatherapy oils</th>
<th>Textures</th>
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<tr>
<td>Patchouli</td>
<td>Masking tape</td>
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<tr>
<td>Geranium</td>
<td>Faux fur</td>
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<td>Ylang ylang</td>
<td>Sandpaper</td>
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<td>Lavender</td>
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<td>Tea-tree</td>
<td>Cardboard</td>
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<td>Tangerine</td>
<td>Packing tape</td>
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<tr>
<td>Musk</td>
<td>Velcro</td>
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<tr>
<td>Vanilla</td>
<td>Suede</td>
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<tr>
<td>Peach</td>
<td>Terry cloth</td>
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<tr>
<td>Peppermint</td>
<td>Carpet</td>
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Fig. 1. A: Illustration of the two different trial types, Target and Distractor, of the forced-choice digging tasks. On Target trials, the rewarded (+) stimulus was the odor-texture bowl, while on Distractor trials, the rewarded stimulus was the blank bowl. B: Illustration of a typical session. On every trial, rats were simultaneously presented with two digging bowls: an odor-texture bowl and the blank bowl. Half of the trials were Target (T) trials and the remaining half were Distractor (D) trials presented in a pseudorandom order. Rats had to use the crossmodal features of the presented odor-texture bowl to determine the correct bowl choice.
2.5. Baseline stimuli

Due to the complexity of our forced-choice digging paradigm, rats were first introduced to the different responses needed for the two trial types, Target and Distractor, without the need for feature binding using two Baseline odor-texture bowls. Rats could use a single odor or texture or the distinct combination of the two features to determine the correct bowl choice as each Baseline stimulus was characterized by a distinct odor and texture. One of the odor-texture Baseline bowls was designated a Target bowl (B1) and the other odor-texture Baseline was designated a Distractor bowl (B2). Although an odor-texture bowl and the blank bowl were present on every trial, on Target trials the rat had to dig in the odor-texture bowl and on Distractor trials the rat had to dig in the blank bowl to retrieve the reward. The same two Baseline odor-texture bowls were used throughout the experiment to measure the effect of lesion on general stimulus discrimination and digging performance.

2.6. Feature-Conjunction stimuli

Each FC stimulus set contained four conjunction odor-texture bowls and the blank bowl. Two of the four odor-texture bowls were designated Target bowls (T1 and T2) and the remaining two odor-texture bowls were designated Distractor bowls (D1 and D2). As illustrated by Fig. 2, crossmodal binding of odors and textures was required to determine the correct bowl choice as each individual odor and texture was associated with both a target and a distractor bowl such that no single odor or texture could be used for correct bowl selection. That is, the crossmodal features of the target and distractor bowls overlapped.

2.7. Feature-Singleton stimuli

The FS stimulus set contained four non-conjunction odor-texture bowls and the blank bowl. Two of the four odor-texture bowls were designated Target bowls (T1 and T2) and the remaining two odor-texture bowls were designated Distractor bowls (D1 and D2). As illustrated by Fig. 2, feature binding was not required to determine the correct bowl choice as each individual odor and texture was associated with either a target or a distractor bowl such that rats could use a single odor or texture or the distinct combination of the two features for correct bowl selection. That is, the crossmodal features of the target and distractor bowls were distinct and did not overlap.

![Feature-Conjunction Stimuli](image1)

![Feature-Singleton Stimuli](image2)

Fig. 2. Illustration of the features defining the Feature-Conjunction (FC) and Feature-Singleton (FS) stimuli. Solid lines indicate pairings of crossmodal features in Target bowls, and dashed lines indicate pairings of crossmodal features in Distractor bowls. For the FC stimuli, feature binding was required for correct bowl selection as each individual odor and texture was associated with both a target and a distractor bowl. For the FS stimuli, feature binding was not required as rats could rely on a single feature (odor or texture) for correct bowl selection as each individual odor and texture was associated with either a target or a distractor bowl.

2.8. Pre-surgical training procedures

2.8.1. Habituation

The testing arena was baited with 2 whole Froot Loops and rats were allowed to explore the apparatus until they consumed at least one of the treats.

2.8.2. Pretraining

For two days, rats were trained to dig for a single whole Froot Loop buried at the bottom of an aluminum metal food bowl in their home cages. Sessions lasted approximately 1 h. The bowls used in their home cages were different from those used during subsequent experimental training in the testing arena; they clipped onto the side of their home cage and measured 4 cm deep x 8 cm in diameter.

2.8.3. General forced-choice digging procedures

At the start of every session, rats were placed in the start box of the apparatus with the sliding door closed. During this time, the appropriate bowl was baited with a single half Froot Loop and placed beside the unrewarded bowl in the testing arena of the apparatus. The blank bowl was always positioned in the far left corner of the chamber with the odor-texture bowl directly in front and flush against the blank bowl to counter the rats’ natural strategy to first dig in the blank bowl and then over trials to acquire when to dig in the odor-texture bowl. The sliding door was then lifted, and the rat was allowed to make a bowl choice, after which it was gently guided back to the start box and allowed to eat the Froot Loop (if obtained) with the door closed. A choice was defined as a dig if one or both paws displaced the bedding of the chosen bowl, or if a rat put its nose half-way down into the bedding. Rats remained in the start box between trials while the bowls were re-baited and replaced, which took on average 30 s. Rats received one session per day. The first few sessions were always discovery sessions, during which rats were allowed to make as many choices as necessary to find the buried reward, but only the first bowl choice counted towards accuracy. During all remaining sessions, rats were only allowed to make a single bowl choice. If rats did not make a choice within 2–3 min, then the Froot Loop was removed and placed on top of the bedding of the rewarded bowl for the rat to find and consume in the start box. In between sessions, any bedding that accumulated in the apparatus was vacuumed up and the walls and floor of the apparatus were wiped down with rubbing alcohol.

2.8.4. Acquisition of Baseline stimuli

Each session consisted of 14 trials, half of which were Target trials and half of which were Distractor trials. Within a session, trials were presented in a pseudorandom order, such that no more than 3 consecutive trials were of the same type (target or distractor). The first three sessions were discovery sessions. Baseline training continued until all rats reached a criterion of at least 6 out of 7 correct Target and 6 out of 7 correct Distractor trials for at least two nonconsecutive sessions.

2.8.5. Acquisition of learning-to-learn Feature-Conjunction stimuli

After the final Baseline training session, in the subsequent session, rats began feature binding training using the learning-to-learn FC stimulus set. FC sessions consisted of 16 trials, the first 4 of which were Baseline trials (2 Target and 2 Distractor trials) identical to those described above. The remaining 12 trials of a session were 6 Target trials (3 T1, 3 T2) and 6 Distractor trials (3 D1, 3 D2) presented in a pseudorandom order, such that no more than 3 consecutive trials of the same type (Target or Distractor) occurred in a session. The first three sessions were discovery sessions and only during training on this initial learning-to-learn stimulus set did rats receive correction trials in which an incorrect response was always followed by the same trial until a correct choice was made. Correction trials did not count towards accuracy.
Fig. 3. Choline acetyltransferase immunohistochemistry of the NBM and MS/VDB. Displayed are a typical sham-lesioned rat on the left and a typical NBM-lesioned rat on the right (magnification 10×). Two schematics of the rat brain in coronal section (A/P stereotaxic coordinates: bregma − 0.84 mm and + 0.60 mm) highlight the NBM and MS/VDB cell-counting frames [50]. The number of ChAT-immunoreactive cells in the NBM (A and B) was significantly reduced in the NBM-lesioned rats (C). There was no significant difference in the number of ChAT-immunoreactive cells between the two lesion groups (D and E) in the MS/VDB (F). ChAT = choline acetyltransferase; MS/VDB = medial septum/vertical limb of diagonal band of Broca; NBM = nucleus basalis magnocellularis.
2.9. Surgery

Rats were assigned to one of two surgical groups, sham-lesion (n = 14) or NBM-lesion (n = 16) equating the groups for pre-surgical Baseline and FC performance. 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. Four 1.0 mm holes were drilled at the following stereotaxic coordinates relative to bregma and the surface of the skull [23]: anterior NBM: Anterior–Posterior (AP) – 0.8 mm, Medial–Lateral (ML) ± 2.5 mm, Dorsal–Ventral (DV) – 8.2 mm; posterior NBM: AP – 1.6 mm, ML ± 2.5 mm, DV – 7.6 mm. In comparison to other excitotoxins, quisqualic acid infused into the NBM results in the greatest reduction of cholinergic input to the neocortex, while causing minimal reductions in cholinergic input to the amygdala and leaving cholinergic neurons in the adjacent pallidal region of the basal ganglia relatively intact [24–28]. There were a total of four intraparenchymal injection sites, two per hemisphere, of 0.8 µl sterile physiological saline (sham lesion) or 5.0 µg µl quisqualic acid (Sigma Aldrich) dissolved in sterile physiological saline through a 26-gauge Hamilton syringe at 0.1 µl/min. The needle was left in place for 4 min after each injection. The body temperature of each rat was maintained with a homeothermic blanket throughout the surgery. After the injections were complete, the wound was closed with staples and EMLA topical analgesic ointment (2.5% lidocaine and 2.5% prilocaine) was liberally applied around the staples. To prevent dehydration, rats were given normal saline (0.9% NaCl; 2 ml/100 g body weight; s.c.) immediately post-surgery. All rats received a minimum of 10 days of recovery with ad libitum food and water before being food restricted for subsequent testing.

2.10. Post-surgical procedures

Experimenter were blind to the surgical group of the animals. All post-surgical FC and FS sessions consisted of 16 trials, the first 4 of which were Baseline trials (2 Target and 2 Distractor trials) identical to those described above to measure the effect of lesion on general stimulus discrimination and digging performance. The remaining 12 trials of a session were 6 Target trials (3 T1, 3 T2) and 6 Distractor trials (3 D1, 3 D2) presented in a pseudorandom order, such that no more than 3 consecutive trials of the same type (Target or Distractor) occurred in a session.

2.10.1. Retrieval of Baseline stimuli

To ensure that rats could still perform the forced-choice digging task and discriminate the odors and textures post-surgery, rats retrieved the Baseline stimuli they had acquired prior to surgery. Baseline retrieval comprised 2 high-accuracy sessions.

2.10.2. Retrieval of Feature-Conjunction (FC) stimuli

After the final Baseline retrieval session, in the subsequent session, rats began retrieval of the learning-to-learn FC stimulus set they had acquired prior to surgery. FC retrieval sessions continued until all rats reached asymptotic performance, which took 6 sessions.

2.10.3. Acquisition of Feature-Conjunction (FC) stimuli

After the final FC retrieval session, in the subsequent session, rats began acquisition of novel FC stimuli. FC acquisition sessions continued until all rats reached asymptotic performance, which took 12 sessions.

2.10.4. Acquisition of Feature-Singleton (FS) stimuli

After the final FC acquisition session, in the subsequent session, rats began acquisition of the FS stimuli. FS acquisition sessions continued until all rats reached asymptotic performance, which took 9 sessions.

2.11. Histological analysis

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 post-fixed 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 and choline acetyltransferase (ChAT) immunohistochemistry. AChE histochemistry was carried out according to methods described elsewhere [23], and was used to assess the extent of cholinergic fiber loss in target structures of the NBM. ChAT immunohistochemistry was carried out according to methods described elsewhere [29,30] to assess the extent of cholinergic cell body loss in the NBM and medial septum/vertical limb of diagonal band of Broca (MS/VDB). After all histological assays, brain slices were mounted on slides, dehydrated and cleared using an ascending ethanol and xylene series, coverslipped with DPX, and examined under a Leica light microscope (DM4000B, Ontario, Canada).

While we were able to complete AChE and ChAT cholinergic assays, due to an unanticipated loss of all of our remaining tissue sections within three weeks of sectioning, we were unable to complete parvalbumin immunohistochemistry to stain for GABAergic cell bodies in the NBM. As stated above, we presume damage to GABAergic cells co-localized in the NBM in our NBM-lesioned rats relative to our sham-lesioned rats. Importantly, both of our cholinergic assays were able to dissociate an intact MS/VDB and its efferents (hippocampus) from selective damage to the NBM and its efferents (frontal and parietal cortices).

2.12. Histological quantification

ChAT-immunoreactive cells were counted in the NBM and MS/VDB as delineated by a rat brain atlas [50] using a Leica light microscope (DM4000B, Ontario, Canada) and Openlab image analysis software (Quorum Technologies, Ontario, Canada). Cell counts for each rat were taken from brain sections at AP = − 0.80 mm relative to Bregma to assess damage to cholinergic cells in the NBM and at AP = + 0.60 mm relative to Bregma to assess damage to cholinergic cells in the MS/VDB. Anatomical landmarks were used to define the borders of the NBM and MS/VDB cell-counting frames; the rectangular outlines superimposed on the rat brain coronal schematics [50] depicted in Fig. 3 delineate the NBM and MS/VDB cell-counting frames used for histological quantification. ChAT-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.

3. Results

3.1. Statistical analysis

All statistical analyses were conducted using SPSS v.14 with an α-level of 0.05.

3.2. Pre-surgical training

Rats required 12 training sessions to reach criterion performance on a Baseline task in which rats learned Target and Distractor trial response classification without the need for feature binding. They
were significantly fewer ChAT-immunoreactive neurons in the NBM of NBM-lesioned compared to sham-lesioned rats [$t(24) = 11.97, p < .001, r^2 = .90; M_{\text{Sham}} = 107.1, SD = 4.6; M_{\text{NBM}} = 27.7, SD = 4.4$. However, there was no significant difference between the number of such neurons in the MS/VDB of the two groups of rats ($t < 1.0, r^2 = .01; M_{\text{Sham}} = 174.6, SD = 5.9; M_{\text{NBM}} = 172.8, SD = 3.6$), indicating that our lesions were specific to the NBM of the basal forebrain. Acetylcholinesterase (AChE) staining revealed a loss of cholinergic fibers in the frontal and parietal cortices, but not the hippocampus of NBM-lesioned rats relative to sham-lesioned rats (Fig. 4).

3.4. Post-surgical testing

3.4.1. Retrieval of Baseline stimuli

Rats in both surgical groups maintained very high accuracy on the Baseline task post-surgery. An analysis of variance (ANOVA) was performed on the Baseline retrieval data using Lesion Condition (sham-lesion or NBM-lesion) as a between-subjects factor and Session as a within-subjects factor. The ANOVA revealed no significant main effect of Lesion Condition ($F < 2.0, r^2 = .05$), a significant effect of Session [$F(3,69) = 8.05, p < .001, r^2 = .26$], and no significant interaction ($F < 2.0, r^2 = .11$).

3.4.2. Retrieval of Feature-Conjunction (FC) stimuli

Fig. 5A depicts post-surgical retrieval of the FC task using the learning-to-learn FC stimulus set rats acquired prior to surgery with data binned into 3-session blocks. An ANOVA was performed on the FC retrieval data using Lesion Condition (sham-lesion or NBM-lesion) as a between-subjects factor and Block as a within-subjects factor. The ANOVA revealed a significant main effect of Lesion Condition [$F(1,23) = 11.62, p < .01, r^2 = .34$] and Block [$F(1,23) = 20.95, p < .001, r^2 = .48$], but no significant interaction ($F < 1.0, r^2 = .02$). An ANOVA on Baseline performance during FC retrieval revealed non-significant effects of Lesion Condition ($F < 2.5, r^2 = .09$) and Block ($F < 1.0, r^2 = .01$), and no significant interaction ($F < 1.0, r^2 = .02$).

3.4.3. Acquisition of Feature-Conjunction (FC) stimuli

Fig. 5B depicts post-surgical acquisition of the FC task using a novel stimulus set with data binned into 3-session blocks. An ANOVA was performed on the FC acquisition data using Lesion Condition (sham-lesion or NBM-lesion) as a between-subjects factor and Block as a within-subjects factor. The ANOVA revealed a significant main effect of Lesion Condition [$F(1,23) = 9.39, p < .01, r^2 = .29$] and Block [$F(3,69) = 31.49, p < .001, r^2 = .58$], but no significant interaction ($F < 1.0, r^2 = .02$). An ANOVA on Baseline performance during FC acquisition revealed no-
significant effects of Lesion Condition ($F<1.0, \eta^2_p = .01$) and Block ($F<1.0, \eta^2_p = .01$), and no significant interaction ($F<2.5, \eta^2_p = .10$).

3.4.4. Acquisition of Feature-Singleton (FS) stimuli

Fig. 5C depicts post-surgical acquisition of the FS task with data binned into 3-session blocks. An ANOVA was performed on the FS acquisition data using Lesion Condition (sham-lesion or NBM-lesion) as a between-subjects factor and Block as a within-subjects factor. The ANOVA revealed no significant main effect of Lesion Condition ($F=3.0, \eta^2_p = .10$), a significant effect of Block ($F(1,56, 35.90) = 66.47, p<.001, \eta^2_p = .74$), and no significant interaction ($F<1.0, \eta^2_p = .03$). An ANOVA on Baseline performance during FS acquisition revealed non-significant effects of Lesion Condition ($F<1.0, \eta^2_p = .001$) and Block ($F<2.0, \eta^2_p = .07$), and no significant interaction ($F<2.0, \eta^2_p = .08$).

4. Discussion

Relative to other neighbouring basal forebrain (BF) nuclei, the nucleus basalis magnocellularis (NBM) provides neuromodulatory input to the neocortex [21], including regions deemed critical for the attentional processes required for feature binding in humans, such as the frontal and parietal cortices [13,14,31]. The NBM has been implicated in attentional processing in non-human animals [15,19,26,32], and the attention-dependent nature of feature binding has been well established by the human cognitive literature [2,4,9,33]. Thus, we hypothesized that the NBM would be a critical brain structure for feature binding.

Findings from the human cognitive literature provide support for the low-attentional load nature of single-feature processing. For instance, patients with attentional deficits who are impaired at encoding conjunctions of features remain fully capable of encoding single features [3–7]. Furthermore, human fMRI studies have revealed selective activation of fronto-parietal networks during tasks requiring feature binding, but not those requiring the processing of single features [9–11]. More recently, it was found that transcranial magnetic stimulation of the intraparietal sulcus influenced the binding of colour and form, but not the detection or processing of single features [8].

Accordingly, we predicted that the single-feature processing of the FS task would demand relatively less attentional resources and would thus be less dependent on the attentional modulatory influences from the NBM than the FC task. Consistent with this hypothesis, NBM-lesioned rats were significantly impaired relative to sham-lesioned rats at acquiring crossmodal Feature-Conjunction (FC) stimuli, while their ability to acquire Feature-Singleton (FS) stimuli remained intact.

Unexpectedly, NBM-lesioned rats were significantly impaired relative to sham-lesioned rats at retrieving the crossmodal FC stimuli they had acquired prior to surgery. Such a retrieval deficit is inconsistent with research suggesting a more crucial role for the NBM in encoding and attention than retrieval and memory [34–38], and it is also inconsistent with our previous cross-species work demonstrating the intact FC retrieval performance of rats and human participants when their attention was challenged [33]. However, there is one important methodological difference between our cross-species study and the present NBM-lesion study. In the former, rats and human participants retrieved a well-learned FC stimulus set following a very short retention interval (1-day or 2–3 min, respectively), while in the present study rats retrieved the FC stimulus set following a 10-day surgical recovery period. It is possible that following such a longer retention interval, a certain degree of re-binding was necessary during retrieval of the FC stimulus set resulting in the poorer retrieval performance demonstrated by the NBM-lesioned rats relative to the sham-lesioned controls. Consistent with such a re-binding hypothesis, the FC retrieval performance of rats from both lesion groups gradually improved across the six sessions of retrieval, suggestive of re-acquisition. Moreover, during the first few sessions of retrieval, sham-lesioned rats were only performing at 70–75% accuracy compared to the very high accuracy levels of 90–95% demonstrated by control rats in our previous cross-species study.

Quisqualic acid infused into the NBM resulted in large reductions in cholinergic input to the neocortex as evidenced by significant cholinergic cell body destruction in the NBM, but not the MS/VDB and loss of cholinergic fibers in the neocortex of NBM-lesioned rats relative to sham-lesioned rats. However, quisqualic acid is a non-selective neurotoxin that destroys neurons irrespective of neurochemical type and cholinergic neurons in the NBM are co-localized with a substantial population of non-cholinergic neurons; y-aminobutyric acid (GABAergic) neurons represent the main population of non-cholinergic neurons in the BF [39–42]. Furthermore, it has been shown that cholinergic drugs (e.g. scopolamine) which act at muscarinic receptors can influence GABAergic neurotransmission [43,44], and recent research has implicated non-cholinergic basal forebrain neurons in attentional processing [45,46]. Such findings suggest the potential contribution of both cholinergic and noncholinergic (e.g. GABAergic) neuromodulation from the NBM to our feature binding findings. For instance, the impaired post-surgical FC retrieval performance of our NBM-lesioned rats may have been due to the quisqualic acid-induced destruction of non-cholinergic (e.g. GABAergic) neurons within the NBM. There is evidence from the non-human animal literature to suggest that while non-selective lesions of the NBM result in both mnemonic and attentional deficits, immunotoxic lesions that selectively destroy cholinergic neurons within the NBM solely result in attentional deficits [47–49].

The findings from the current study provide insight into the functional role of the NBM and establish the importance of this basal forebrain structure to the fundamental cognitive process of feature binding. The NBM provides 90% of the cholinergic input to the neocortex [21] and our previous pharmacology work with this feature binding paradigm has revealed that ACh acting at muscarinic receptors provides the neuromodulatory support for crossmodal [22] and intramodal feature binding [33]. The present excitotoxic lesion findings thus permit us to probe the neurochemical underpinnings of the NBM’s role in feature binding using the cholinergic-selective immunotoxin 192 IgG-saporin to determine whether it is cortical cholinergic input from the NBM that makes this basal forebrain region essential for feature binding. Future work must also concentrate on delineating the specific neocortical targets (frontal vs. parietal cortices) NBM efferents must reach for successful feature binding to occur.

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