The Role of Cortical Cholinergic Inputs in a Selective Attentional Suppression Task

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Abstract

Visual stimuli are in constant competition with one another due to the limited processing capacity of the visual system. As a result, selective attention is necessary to suppress distractor stimuli and enhance perceptual processing of target stimuli. Cholinergic projections from the nucleus basalis magnocellularis (NBM) in the rat basal forebrain to the prefrontal (PFC) and posterior parietal (PPC) cortices play a significant role in the modulation of selective attention. These two cortical areas amplify the signals of the attended stimulus in order to overcome those elicited by the unattended stimulus, thereby increasing the signal-to-noise ratio. In the present study, rats performed a learning-to-ignore task where they learned to respond to a stimulus that they had previously ignored in two different contexts. The cholinergic neurons of the NBM were lesioned with the selective cholinergic immunotoxin 192 IgG-saporin (SAP) to reduce cholinergic cortical afferentation. Selective attention was impaired in the SAP group; this was evidenced by the SAP rats’ better performance in the learning-to-ignore task. As hypothesized, the reduction in cortical levels of acetylcholine (ACh) impaired the ability to ignore distractor stimuli and improved performance. The impaired selective attention demonstrated by the SAP group is comparable to that shown in older adults and patients with Alzheimer’s disease. The present study confirms the role of ACh in attentional modulation, additionally, it suggests that treatment to improve attentional processing should include therapeutic strategies that increase the level of cholinergic neurotransmission in the central nervous system.

Introduction

Selective attention refers to the ability to focus on a pre-determined target, or behaviourally-relevant stimulus while simultaneously ignoring the distractor or behaviourally-irrelevant stimulus [1]. Selective attention resolves competition between visual stimuli by increasing the firing rate of cells whose receptive fields contain the attended stimulus (i.e. amplifying the signals of the attended stimulus) [2] and suppressing those of the unattended stimulus. This increases the salience of the attended stimulus relative to stimuli that are being ignored [3]. Furthermore, this biased competition model of attention holds true even when the distractor stimulus is naturally more salient than the target stimulus, known as bottom-up bias [3]. As bottom-up processes can interfere with one’s ability to process target visual information, top-down mechanisms are necessary to suppress irrelevant stimuli. This is especially true in tasks that are more attentionally demanding as there are more distractor stimuli that would otherwise directly attention away from the target stimulus.

It is hypothesized that selective attention is modulated by acetylcholine (ACh), a neurotransmitter synthesized by cholinergic neurons of the central nervous system. These cholinergic cells originate in the basal forebrain cholinergic complex, more specifically, the nucleus basalis magnocellularis (NBM), as it is known in rats, or in the nucleus basalis of Meynert, as it is known in humans [4,5]. These cholinergic neurons modulate cortical levels of ACh by projecting to the prefrontal (PFC) and parietal (PPC) cortices [4].

As a task becomes more attentionally demanding, cholinergic neurons innervate neurons of the PFC to increase the salience of an attended stimulus [6]. The PFC in turn signals to the PPC, known to be involved in the suppression of irrelevant stimuli [7]. A study conducted by Gills, Sarter and Givens [8] found that during a sustained attention task, cholinergic deafferentation of the rat PFC decreased PFC firing rates associated with the appearance of a visual distractor. This study suggests that a rise in ACh levels in the PFC correlates with neuronal activity associated with higher demands for attentional processing. Furthermore, the PFC has the ability to increase ACh efflux in the PPC when a task is attentionally demanding [9]. Nelson et al [9] found that when carbachol, a cholinergic agonist, was infused into the PFC, ACh efflux in the PPC increased. When carbachol was infused into the PPC however, no change in ACh efflux in the PFC was found. This study demonstrates that the PFC has the unique role of mediating top-down regulation by modulating ACh release in the PPC.

Given the significant role that ACh has on attentional modulation, it is not surprising that the degeneration of cholinergic neuronal cells in the basal forebrain has a huge impact on overall cognitive decline. Reduced cholinergic innervation to the frontal lobe of patients with Alzheimer’s disease, for example, was found to be positively correlated with memory impairment [5]. This may be due in part to their reduced ability to inhibit irrelevant stimuli, which in turn compromises their ability to successfully encode relevant stimuli [10].
When the rat touched the target stimulus, the circular light at the rear wall lit up touchscreen monitor. When the rat touched the target stimulus, the circular light at the rear wall lit up. This signalled to the rat that water had been dispensed into the water-well. When the rat touched the distractor stimulus, a white light appeared on the screen indicating to the rat that water had been dispensed into the water-well. When the rat touched the target stimulus, the circular light at the rear wall lit up. This signalled to the rat that water had been dispensed into the water-well.

Figure 1: Progress of a rat within the operant chamber during Learning to Learn, Prime 1, Prime 2, and Probe. (a) Prior to presentation of the two-second tone and one-second delay, no stimuli were displayed on the touchscreen monitor. (b) When the rat touched the target stimulus, the circular light at the rear wall lit up. This signalled to the rat that water had been dispensed into the water-well. (c) 0.05 ml of water was dispensed upon correct selection of the target stimulus. (d) When the rat touched the distractor stimulus, a white light appeared on the screen indicating to the rat that it had made an error. No water was dispensed into the water-well.

Figure 2. (a) Illustration of the visual stimuli used in each block during pre-surgical training. (b) Illustration of the visual stimuli used in each block during post-surgical training.

The following visual operant attentional suppression study demonstrates that the lesioning of cholinergic neurons in the NBM improves the performance of rats in a two contexts learning-to-ignore task [11], i.e., reduces the rats’ ability to attentionally suppress task-irrelevant stimuli. In this learning-to-ignore task, rats repeatedly ignored a distractor visual stimulus in two separate stimulus contexts and then responded to the same visual stimulus as a target in a new stimulus context. The cholinergic cells of the NBM were lesioned with the selective cholinergic immunotoxin 192 IgG-saporin (SAP) in one set of rats to determine whether or not they are involved in selective attention. This reduced the number of cholinergic cells in this region and as a result, lowered cholinergic innervation to the PFC and PPC. With a lower cholinergic fiber density in these cortical areas, the SAP group was unable to learn to ignore the distractor stimulus to the same degree as the sham-lesioned control group. As a result, when the previously-ignored distractor stimulus became the target stimulus, the SAP surgical group was expected to perform better compared to the control group.

Methods and Materials

Subjects

The subjects used for this study were twenty male experimentally naïve Long-Evans rats (Charles River, Montreal, Quebec). As one of the rats passed away, the total number of rats used in the study was reduced to nineteen. At the beginning of the experiment, rats were 8 weeks of age and weighed approximately 200 g. Rats were housed individually in 45 cm long x 25 cm wide plastic clear tub cages. Food was restricted to 5 pellets per day and the vivarium was temperature and humidity controlled. Rats were maintained on reversed 12 h light/dark cycle (lights off at 8 A.M) and training was conducted during the dark phase of the light/dark cycle between the hours of 8 A.M and 8 P.M 6 days a week. 2 weeks prior to the start of the experiment, rats were handled 10 minutes per day for 6 days. Water was available ad libitum during this time. During the 24 h period prior to training, rats were water deprived. Following training, rats had access ad libitum to water for 20-30 minutes per day. This study was approved by the University of Toronto’s Local Animal Care Committee.

Apparatus

The equipment used for this study is described in Botly and De Rosa (2011) [12].

Visual Stimuli

The visual stimuli were black-and-white computer generated shapes that consisted of one target stimulus and one distractor stimulus. The target and distractor stimuli varied between pre-surgery (Figure 2A) and post-surgery (Figure 2B).

Pre-Surgical Training Procedures

Variable Light Water

Rats underwent a positive reinforcement light-water task where they learned to associate the lighting of a circular light above the watering well with the dispersion of 0.05 ml of water. This was done for 30-minute sessions a day.

Touch Light Water

Rats underwent a positive reinforcement touch-light-water task where they were required to learn to associate touching the touchscreen with the lighting of the circular light above the watering well. Water was available ad libitum during this time. This was done for 72 trials per session or 50-minute sessions, whichever came first, until a pre-determined criterion was met (an average reaction time of 5 seconds for 2 consecutive days).
Single Stimulus
In this task, rats were required to touch a single white square stimulus presented in the middle of the touchscreen. Rats learned to associate touching the stimulus with access to 0.05 ml of water. This was done for 72 trials per session or 50-minute sessions, whichever came first, until a pre-determined criterion was met (an average reaction time of 5 seconds for 2 consecutive days).

Moving Stimulus
This task is essentially the same as the single stimulus task, except that the single white square stimulus was now presented on either the left or right bottom of the screen. This was done for 80 trials per session with no time limit.

Learning to Learn
In this task, rats were required to discriminate between a target stimulus and a distractor stimulus. Touching the target stimulus was positively reinforced with gaining access to 0.05 ml of water. Touching the distractor stimulus was negatively reinforced with the emission of ten seconds of bright light from the touchscreen (Figure 1). This was done for 80 trials per session, without a time limit, until a pre-determined criterion was met (18 correct responses within 20 trials at any point during the session).

Learning-to-ignore – Two contexts (LI)
The conditions consisted of three blocks, namely prime 1, prime 2 and probe (Figure 2A and Figure 2B). Each block was run for ten days. Eighty trials were performed per session with no time limit. In prime 1, rats had to attend to stimulus A and ignore stimulus B. In prime 2, stimulus B was to be ignored while the rats attended to a novel stimulus C. The addition of a novel stimulus to prime 2 created a second context in which stimulus B was to be ignored. This increases attentional suppression of stimulus B, making it more difficult to attend to B in the probe block [11]. In the probe block, rats now attended to stimulus B while ignoring a novel stimulus D.

Surgical Procedure
Rats were divided into two surgical groups, namely sham-lesion (n=8) and NBM-lesion (n=11) (SAP). The two groups were matched based on pre-surgical performance to ensure that any difference in performance between groups observed in the post-surgery probe block was due to the lesion of the NRM and not due to pre-existing individual differences. Surgeries were performed under aseptic conditions. Immediately prior to the surgery, rats received an intraperitoneal injection of atropine (0.05 mg/kg) to prevent fluid buildup in the lungs. Rats were anesthetized with isoflurane (approximate maintenance dose was 2% with 1 L/min of oxygen). Rats in the sham lesion group received 0.2 μL of sterile 0.1M phosphate-buffered saline (PBS). Rats in the SAP group received 0.2 μL of 192 IgG-saporin in sterile 0.1 M PBS (concentration of saporin = 0.3 mg/ml). Injections were given at the following stereotaxic coordinates relative to bregma: anterior NBM: Anterior–Posterior (AP)— 0.8 mm, Medial–Lateral (ML)±2.6 mm, Dorsal–Ventral (DV)— 7.8 mm; posterior NBM: AP—1.3 mm, ML ±3.0 mm, DV—7.3. Four injections were given altogether, two per hemisphere at a rate of 0.1μL/min. After each injection, the needle was left in place for 3 minutes before being removed. 20 minutes prior to the end of the surgery, rats were given subcutaneous injection of analgesic ketoprofen (5 mg/kg) as well as the injection of 3ml of warm, sterile saline. Staples were used to close the wound and EMLA topical analgesic ointment (2.5% lidocaine and 2.5% prilocaine) was applied around the staples. Rats were given a minimum of 10 days to recover with ad libitum food and water.

Retrieval of Probe stimuli
After the rats had fully recovered, the probe block of the learning-to-ignore task was run for three days using pre-surgery visual stimuli. The performance of the rats on these three days was compared to that of the last day of pre-surgery probe to ensure that the surgery did not interfere with their ability to perform the task.

Post-Surgical Training
Experimenters were blind to the surgical group of the rats. Each block was run for ten days as in the pre-surgical training. Different stimuli were used for each block in order to avoid interference from pre-surgical training.

Learning-to-ignore – Two contexts (LI)
Rats were once again trained in a learning-to-ignore task using the same three blocks used in pre-surgical training, namely prime 1, prime 2 and probe. Different visual stimuli were used for all blocks in order to avoid interference from pre-surgical training. Each block was run for 10 days. Eighty trials were performed per session with no time limit.

Histological Analyses
Isoflurane (3-4%) was used to anesthetize the rats who were subsequently transcardially perfused with 150 mL of saline followed by 150 mL of ice-cold 4% paraformaldehyde. The brains were extracted from the skull and immediately postfixed in 4% paraformaldehyde for 2h at 4°C. Brains were sectioned at a thickness of 60 μm using a cryostat equipped with a freezing-sliding microtome (Leica Microsystems). Choline acetyltransferase (ChAT) immunohistochemistry was performed to determine whether there was a reduction of cholinergic cells in the NBM (Figure 4). To confirm that 192-IgG saporin did not affect other cells in the NBM, namely GABAergic cells, parvalbumin immunohistochemistry was conducted according to the methods described in Baxter et al [13] and De Rosa et al [14] (Figure 5). To
confirm that there was no reduction of cholinergic cells in other areas of the basal forebrain, namely the medial septum (MS) and vertical limb of the diagonal band of Broca (VDB), ChAT immunohistochemistry was performed (Figure 6). Lastly, acetylcholinesterase (AChE) histochemistry was performed according to the method described by Paxinos and Watson [15]. This was done to determine whether cholinergic fiber loss occurred in the PFC (Figure 7) and PPC (Figure 8). Once the histological assays were completed, brain slices were mounted on slides, dehydrated, and cleared with the use of an ascending ethanol and xylene series. Brain slices were coverslipped with the histological mountant distyrene plasticizer xylene. Slices were then examined under a Leica light microscope (DM4000B).

**Histological Quantification**

The methods used for cell counting and AChE densitometry are described in Botly and De Rosa [16].

**Statistical Analysis**

Performance on the visual operant attentional suppression study was measured using percent accuracy. The data from all rotations were compiled to form a complete data set and the average performance accuracy across all 19 rats for each block was calculated. Statistical analyses were conducted using SPSS version 17.1 software and a 95% confidence level was used to measure significance.

**Results**

**Retrieval of Probe Stimuli**

An independent samples t-test comparing the mean accuracy scores of control and lesion rats on the last day of pre-surgery probe revealed no significant difference between the two groups (t(17) = 0.41, p > 0.05). A between subjects mixed-design repeated measures ANOVA comparing the post-surgery performance of control and SAP rats on the three days of pre-surgery probe yielded no significant group effect [F(1, 17) = 1.309, p > 0.05].

**Performance during the Probe Block**

A between subjects mixed-design repeated measures ANOVA was run for each block to compare the performance of the control group to that of the SAP group. For prime 1 and prime 2, no significant difference was found between groups [F(1,17)=0.856, p>0.05, M_sham = 66.5%, M_NBM = 62.0%; F(1,17)=1.939, p>0.05, M_sham = 79.4%, M_NBM = 72.4%, respectively]. In the probe block however, a significant difference was found [F(1,17)= 6.381, p<0.05, M_sham = 27.9%, M_NBM = 34.9%]. Two-tailed t-tests were also run for each day of post-surgery probe to see if there was a significant difference in performance between the two groups. A significant difference between groups was found on day four [t(17)=-2.283, p<0.05], day five [t(17)=-2.769, p<0.05], day six [t(17)=-3.045, p<0.01] and day seven [t(17)=-2.441, p<0.05] of probe (Figure 3).
NBM \([t(17)=7.73, p<0.0001]\), and the cholinergic fiber density of the PFC \([t(10)=-9.31, p<0.0001]\) and PPC \([t(11.45)=4.74, p<0.01]\). There was no significant difference between groups in the number of cholinergic neurons counted in the MS/VDB \([t(15.59)=-0.08, p>0.05]\) or the number of GABAergic neurons counted in the NBM \([t(14.69)=-0.26, p>0.05]\).

**Discussion**

**Performance on Learning-to-ignore Task**

As expected, rats in the SAP group performed significantly better relative to the control group in the probe block. In contrast, there were no significant performance differences during the prime blocks. By lesioning cholinergic neurons in the NBM, cholinergic deafferentation to the PFC and PPC was reduced. The resulting cholinergic deafferentation in these cortical areas impaired the ability of these rats to ignore distractor stimulus B in both the prime 1 and prime 2 blocks. As a result, the impaired attentional suppression of this stimulus during the prime blocks allowed the SAP group to attend to stimulus B better than the control group when it became the target stimulus during the probe block. The control group demonstrated a comparable learning-to-ignore pattern of behaviour to human participants i.e., learning to ignore stimulus B in two different contexts severely compromised their ability to attend to this stimulus during the previously-unattended probe block [11].

**Histological Findings**

There was a significant reduction in the number of cholinergic neurons only in the NBM. This confirms that the 192-IgG saporin was indeed injected into the target area and that other areas of the basal forebrain were not affected. The loss of cholinergic fiber density in the PFC and PPC further confirms that there was significantly less cholinergic innervation to these cortical areas, resulting in impaired selective attention.

**Clinical Relevance**

Our findings demonstrate the importance of ACh in the modulation of selective attention. As loss of cholinergic innervation to the cortex and hippocampus is a marker for Alzheimer’s disease, possible avenues for the treatment of this neurodegenerative disease may lie in therapeutic strategies that increase cholinergic neurotransmission and prevent cholinergic cell loss in these brain areas [17]. Such examples of prospective therapeutic agents include neurotropins such as nerve growth factor (NGF) and brain derived growth factor (BDNF). NGF and BDNF are widely accepted as essential signaling factors for cholinergic neuronal differentiation as well as for the maintenance of the cholinergic phenotype [18]. Mouse embryo studies have shown that these growth factors increase messenger RNA expression of cholinergic markers, namely choline acetyltransferase (ChAT), acetylecholinesterase (AChE), choline transporter (CHT) [19, 20] and vesicular acetylcholine transporter (VACHT) [21]. The use of NGF and BDNF in the treatment of Alzheimer's disease is yet to be practiced in a clinical setting.

**Conclusion**

The present study demonstrates that reducing cholinergic input to the PFC and PPC by lesioning the NBM improves performance of rats in a learning-to-ignore task; this improvement reflects impaired attentional suppression of previously-ignored distractors. Our study is the first of its kind to use touchscreen behaviour in rats to demonstrate that the selective cholinergic lesioning of the NBM is sufficient to impair selective attention. Pharmacological interventions that reduce the loss of cholinergic neurons or increase cholinergic neuronal function may be considered in the treatment of attentional deficits associated with neurodegenerative diseases such as Alzheimer's disease.

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**References**