Published in final edited form as:

Behav Neurosci. 2007 February ; 121(1): 80–89.

Disconnection of the amygdala central nucleus and the substantia innominata / nucleus basalis magnocellularis disrupts performance in a sustained attention task

Peter C. Holland

Johns Hopkins University

Abstract

The basal forebrain cholinergic system is broadly implicated in the regulation of attention. Disruptions in the function of this system produce impairments in many attentional functions, including the performance of well-learned responses under increased attentional load, and the surprise-induced enhancement of learning rate. Similarly, lesions of the amygdala central nucleus (CeA) have been found to impair attentional function in some circumstances. Here we examined the effects of lesions that disconnected CeA from the cholinergic substantia innominata/nucleus basalis magnocellularis (SI/nBM) on performance in a modified five-choice serial reaction time (5CSRT) task, thought to assess selective or sustained attention. The lesions impaired performance under conditions of increased attentional load, suggesting that a circuit that includes CeA and SI/nBM regulates these aspects of attention.

Keywords

amygdala; attention; cholinergic system; nucleus basalis; substantia innominata

Considerable evidence implicates the basal forebrain cholinergic system in the regulation of attention (e.g., Sarter & Bruno, 1997; Everitt & Robbins, 1997). Cholinergic lesions of the substantia innominata/nucleus basalis magnocellularis (SI/nBM) and the administration of anticholinergic drugs compromise performance in a number of tasks thought to assess attention, including covert orienting (Voytko, 1996), cue validity (Chiba et al., 1999), signal detection (McGaughey & Sarter, 1995; McGaughey et al., 1996), five-choice serial reaction time (5CSRT) (e.g., McGaughey et al., 2002), and several Pavlovian conditioning tasks that assess changes in conditioned stimulus (CS) associability (the ease or rate with which a cue may enter into new associations; Pearce & Hall, 1980) after the induction of surprise (e.g., Bucci, et al, 1998; Chiba et al., 1995; Holland & Gallagher, 2006).

Gallagher and Holland (Gallagher & Holland, 1992; Holland & Gallagher, 1999) asserted that in many circumstances the action of SI/nBM is modulated by the amygdala central nucleus (CeA). For example, in studies of surprise-induced enhancement of cue associability, we found that rats with bilateral lesions of the CeA (Holland & Gallagher, 1993) and rats with lesions that functionally disconnect CeA from SI/nBM (Han et al., 1999), show impairments similar

Contact: Peter C. Holland, Department of Psychological and Brain Sciences, 3400 North Charles St, Baltimore MD 21218 USA, Voice: 1 410 516 6396, FAX: 1 410 516 0494, Email: pch@jhu.edu.

Publisher's Disclaimer: The following manuscript is the final accepted manuscript. It has not been subjected to the final copyediting, fact-checking, and proofreading required for formal publication. It is not the definitive, publisher-authenticated version. The American Psychological Association and its Council of Editors disclaim any responsibility or liabilities for errors or omissions of this manuscript version, any version derived from this manuscript by NIH, or other third parties. The published version is available at <http://www.apa.org/journals/bne/>

to those shown by rats with bilateral cholinergic lesions of SI/nBM (Chiba et al., 1995). Similarly, Holland et al. (2000) found that bilateral lesions of amygdala central nucleus CeA impaired attentional performance in a simplified, 3-choice version of the 5CSRT task. In the present study, we extended these observations by considering whether performance in a 5CSRT task is also affected by lesions that functionally disconnect CeA from SI/nBM (Experiment 2).

We also examined the effects of cue validity in this task, by comparing responding to cues that consistently signaled the availability of food to responding to cues that predicted food availability on only 50% of the trials. Consistent with several behavioral theories of attention in associative learning (e.g., Mackintosh, 1975) it seems intuitive that reliable predictors of important events should capture attention more readily than unreliable predictors of those events, and thus be more resistant to the challenges to attention used in 5CRST tasks. By contrast, the Pearce-Hall model (1980) postulates that the associability of a CS present within a learning trial is enhanced if the outcome of that trial is surprising, and reduced if the outcome is expected. Thus, within this model, attention (for purposes of acquiring new learning) is maintained at a higher level to cues that are relatively poor predictors of their consequences (and which are therefore accompanied by surprise) compared to cues that are reliable predictors of their consequences (and which are thus not accompanied by surprise). This contention is supported by a great deal of behavioral data from a variety of conditioning procedures (e.g., Holland, 1997, 2005; Pearce & Hall, 1980). The question of interest here is whether partially-reinforced cues might also command more attention in the 5CSRT task and hence be more resistant to attentional challenges than consistently-reinforced cues.

Accordingly, in Experiment 1 we examined, in intact rats, the effects of imposing partial reinforcement contingencies on responding to some of the cues in a multiple-cue task after prior consistent reinforcement of those cues. Consistent with theories such as Mackintosh's (1975), we found that responding to partially-reinforced cues was especially sensitive to attentional challenges produced by shortening cue duration. In Experiment 2, we sought to determine whether CeA exerts control over attention in this 5CSRT task via its interaction with the basal forebrain cholinergic system, as we have found to be the case in tasks that measure associability changes (Han et al., 1999). Thus, we examined the effects of lesions that disconnected CeA from SI/nBM on performance in this task. Although baseline performance was unaffected by disconnection of these two brain regions, performance under conditions of reduced cue duration was substantially impaired.

Experiment 1

In Experiment 1, all rats were first trained with 5CSRT procedures, in which nose pokes to a briefly-illuminated (500 ms) port in an array of 5 ports were reinforced with food delivery on every trial (consistent reinforcement, CRF). Then, we introduced partial reinforcement (PRF) contingencies for 2 of the port cues such that correct responses to those ports were reinforced on only half of the trials on which they were presented. Next, we assessed the effects of a cue duration challenge, in which the duration of the cues was reduced first to 250 ms and then to 100 ms. If PRF cues control more attention than CRF cues in this task, as they do in tasks that measure rates of new learning about cues (Collins & Pearce, 1985; Collins et al., 1983; Kaye & Pearce, 1984), then responding to the PRF cues should be less susceptible to the cue-duration challenge. By contrast, if rats devote fewer attentional resources to PRF cues than to CRF cues, then responding to PRF cues should be more susceptible to that challenge.

The variant of the 5CRST task that we used (prior to the introduction of PRF contingencies) was identical to that used by Holland et al. (2000) except that 5 rather than 3 stimulus-ports were available. Notably, this variant differs in many respects from the 5CRST procedures used by most other investigators. First, in our procedure, except for the food reinforcer, the delivery

of all events is independent of the rat's behavior. By contrast, in a typical 5CSRT protocol, each subject's behavior influences the initiation and termination of trials. For example, trials are typically postponed or canceled if nose-pokes are performed prematurely (prior to cue illumination). Second, whereas in typical 5CSRT protocols, an error terminates the trial without opportunity to perform the correct response, errors had no such consequence in our procedure. Nevertheless, we have found the variant we use in the present study to be sensitive to cue-duration challenges and CeA lesions in other studies (Holland et al., 2000), and thus it seems appropriate to use in the current study. We consider possible effects of these procedural differences further in the discussion.

Materials and methods

Subjects—Sixteen male Long-Evans rats (Charles River Laboratories, Raleigh, NC), initially weighing 325–350 g, were individually housed in a climate-controlled vivarium on a 14:10-hr light/dark cycle (lights on at 07:00) with free access to water. They were fed ad libitum during acclimation to the vivarium and during postoperative recovery periods (Experiment 2) but throughout the remainder of the experimental training, they were given limited access to food to maintain their weights at 85% of free-feeding weights.

Apparatus—The behavioral training apparatus consisted of four individual 5CSRT chambers (25 × 25 × 25 cm; Cambridge Cognition, Cambridge, UK). Each chamber had aluminum front and side walls and a clear acrylic back wall and top. Five 2.5 × 2.5 × 4 cm (deep) stimulus/response ports were spaced 2.5 cm apart, and centered on the front, curved wall of the chamber, 2 cm above a grid floor. A 3w lamp at the back of each port provided port cues; responding in the ports was detected with infrared phototransistors. A recessed food cup was mounted in the center of the back wall of the chamber, and was fitted with a lamp that was illuminated when food pellets were delivered, and with a transparent flap to detect food cup entries. A 3w overhead lamp mounted at the center of the chamber ceiling could be illuminated as a ready signal to indicate trials with the port cues. Each chamber was enclosed in a sound-attenuated box where ventilation fans provided masking noise (70 dB). A bank of infrared LEDs provided background illumination for video monitoring and recording, but this illumination was invisible to the rats. A television camera was mounted within each box and images were recorded during behavioral training and testing.

Procedures—The rats were first familiarized with the apparatus in four sessions. In the first 30-min session, food pellets (45 mg, formula PJAI; Research Diets, Lancaster, New Hampshire) were present in the food cup, and the acrylic flap to the food cup was propped open. In the next 30-min sessions, food pellets were again placed in the food cup, but the flap door was not propped open. In addition, all five port lights were continuously illuminated, and food pellets were placed in each response port as well as in the food cup. In each of the next two 30-min sessions, there were 16 deliveries of a food pellet, accompanied by a 1-s illumination of the food cup light, to train the rats to collect food from the food cup.

In the baseline task used here, the beginning of a trial was signaled at random intervals by the illumination of the overhead lamp ready signal. After a constant 5-s ready period, one of the five target ports was illuminated for 500 ms. Each port was equally likely to be illuminated on any trial. The first response to the correct port within 5 s of port illumination was reinforced with the delivery of a food pellet to the food cup (accompanied by a 1-s illumination of the food cup) and the darkening of both the port (if still illuminated) and the ready signal. If no correct response was made before the end of the 5-s response window, the ready signal was darkened and the trial ended. Responses to the ports that were not illuminated on a trial were recorded as errors; errors had no scheduled consequences. Sixty trials were presented in random order at predetermined intervals within each 30-min session; trial delivery was not affected by

the rats' behavior. Each rat received two sessions daily, the first around 7:00 a.m. and the second around 3:00 p.m.

Rats were shaped to this procedure gradually, but all rats received the same treatment (the shaping was not individualized). Between sessions, the duration of port illumination was reduced and the number of trials increased, from 30 s (6 of each port cue per session) in the first three training sessions to 500 ms (12 of each port cue per session) over the course of 13 sessions. When the target duration was 5 s or more, responses were effective throughout the illumination of the target stimuli. After 5 sessions with 500-ms port cue durations, the final reinforcement contingencies were introduced. For each rat, two of the ports were designated for consistent reinforcement (CRF), two for partial (50%) reinforcement (PRF) and one for no reinforcement (extinction, EXT). For each rat, the EXT port was the center port; the two CRF ports were on one side of the array and the two PRF ports on the other side (counterbalanced). Each session included 12 trials with each port cue, as before. CRF trials were identical to those presented previously. Responding on half of the trials with the PRF port cues was reinforced as before, but on the other half of those trials, correct responses terminated the ready signal but did not produce food delivery or food cup illumination. On trials with the EXT port cues, "correct" responses had the same consequences as errors.

After 9 CRF/PRF training sessions, all rats received a pair of attentional challenge sessions, to determine the effects of the partial reinforcement contingencies on the allocation of attention. In these challenge sessions, the port cue duration was reduced to 250 ms and 100 ms, respectively; otherwise these sessions were identical to the training sessions. Next, the rats were returned to the original 500-ms target condition for 2 sessions that were identical to the final baseline training sessions.

Data analysis—Behavioral performance was assessed with several measures. The primary measures were the percentage of trials on which at least one correct response occurred during the 5-s interval after port light onset, and the percentage of trials on which at least one error occurred. Errors were defined as entries into ports other than the one illuminated. In addition, we recorded several measures of port entry response latency (time since onset of the port light), premature responses (port entries that occurred during the ready signal but before onset of a port stimulus), and perseverative responses (port entries that occurred after food delivery), for both correct and error responses.

Holland et al. (2000) used a composite latency measure as their primary index of performance. This measure was influenced somewhat by the accuracy of responding as well as the speed of responding: if no correct response occurred on a trial, a maximum latency value of 5 s, the response window duration, was assigned. Although this measure was sensitive to manipulations of attention, in the present study we elected to use the percentage of trials with a correct response as our primary measure, for several reasons. First, the composite measure is seldom reported by other investigators who use the 5CSRT procedure. More important, the percentage response measure is more informative for the key experimental variable in this study, reinforcement probability, because it also specifies the actual reinforcement probabilities in different experimental conditions. Thus, rats that responded correctly on 90% of PRF trials actually received food on 45% (rather than the scheduled 50%) of those trials. Finally, in both the present study and that of Holland et al. (2000), statistical analyses showed similar results for both measures.

Separate analyses of variance (ANOVAs) were conducted for each of the response measures, followed by planned individual comparisons and post hoc tests using the Tukey honest significant difference (HSD) procedure. The level of significance adopted was $p < 0.05$.

Results

Over the course of the initial 17 training sessions, all rats acquired and maintained correct responding despite the decreasing duration port cues. Introduction of the PRF contingency for half of the port cues had no immediate effect on responding, although differences between responding to CRF, PRF, and EXT cues were observed later.

The black bars in Figure 1 show responding to the CRF and PRF cues in the final two sessions of training, which provided a baseline for the challenge tests. The rats displayed more correct responses (Figure 1A) and fewer errors (Figure 1B) to the CRF cues than to the PRF cues. In two separate cue duration challenge sessions, the duration of illumination of the port light was reduced from its training level of 500 ms to 250 ms (light gray bars in Figure 1) and 100 ms (dark gray bars), respectively. Relative to baseline responding, the rats made fewer correct responses and more errors in the challenge sessions, supporting the use of this manipulation as a means to provide an added demand on attentional processing. As in the baseline training sessions, correct responding was more frequent and errors less frequent on CRF trials than on PRF trials. Furthermore, the reduction of target cue duration reduced correct responding to the PRF more than it disrupted performance to the CRF cues. Thus, CRF cues controlled more attention than PRF cues. By contrast, reductions in cue duration increased error responding to CRF and PRF cues similarly.

The data from the baseline and challenge sessions were subjected to reinforcement contingency (CRF or PRF) X cue duration (500, 250, or 100 ms) ANOVAs. ANOVAs of correct and error responses were conducted separately. There were significant main effects of contingency for both correct responses, $F(1, 12) = 57.41$, $p < .001$, and errors, $F(1,12) = 7.42$, $p = 0.018$. Likewise, there were significant main effects of cue duration for both correct responses, $F(2,24) = 61.14$, $p < 0.001$, and errors, $F(2,24) = 12.86$, $p < 0.001$. A planned contrast of correct CRF and PRF responding on baseline sessions with responding on challenge sessions showed that the superiority of CRF responding over PRF responding was greater in the challenge tests, $F(1, 12) = 15.44$, $p = .002$. A comparable contrast of errors was not significant, $F < 1$, $p = 0.460$.

The training and test sessions also included presentations of the EXT (center) cue, to which responses were never reinforced. Although baseline (500-ms cue) correct responding was lower to the center EXT cue than to either the CRF or PRF cues in every rat, both correct and error responses to the EXT port showed considerable resistance to extinction. The percentages of trials with a “correct” (EXT port) response were $74.4 \pm 5.0\%$, $69.8 \pm 7.8\%$, and $73.5 \pm 7.9\%$ in the 500-ms, 250-ms, and 100-ms sessions, respectively. The corresponding percentages of trials with at least one error were $99.0 \pm 1.0\%$, $93.8 \pm 3.4\%$, and $96.9 \pm 3.0\%$, respectively. An analysis of errors (Table 1) showed that on EXT trials, the rats were as likely to respond to the (unilluminated) CRF or PRF ports as to the “correct” (illuminated) EXT port. By contrast, on CRF and PRF trials, rats were equally likely to respond in error to the unilluminated CRF, PRF, or EXT ports, $F < 1$, $p = 0.675$, but considerably more likely to respond to the correct (illuminated) port. These differences in response distribution were maintained in the challenge sessions as well. Furthermore, unlike with the CRF and PRF cues, the cue duration challenges did not reliably affect the frequency of either correct or error responses on EXT cue trials, $F < 1$, $p = 0.565$. Thus, the EXT cue apparently did not exert powerful stimulus control over responding. In other words, although CRF and PRF cues captured attention, directing responding toward the illuminated cue light, the EXT cue did not selectively direct responding. Such randomly-generated responding might not be expected to be affected by variation in cue duration.

Other response measures—Most investigations of 5CSRT performance report sensitivity of a variety of response measures to procedural and brain manipulations. Consistent with those studies, we found that the latency of correct responding showed similar patterns of data and

statistical significance as the percentage of correct responding measure. However, the sensitivity of many of these other measures is related to the penalties exacted for inappropriate responses in typical 5CSRT procedures. For example, because trials are canceled if cue port responding occurs prematurely (before port illumination) or if errors are made, rats trained on typical 5CSRT procedures are prone to make few premature responses and few errors, but omit responding on a substantial portion of trials. These behaviors are often sensitive to brain lesions, and this can provide considerable information about brain function. However, because there was no penalty for either premature or error responses in our task, response omissions in these experiments were rare (fewer than 5% of trials across all conditions) and premature responses were common. Premature responding showed a significant, $F(2, 24) = 5.99$, $p = 0.008$, spatial bias: responding to the center port was $53.8 \pm 6.9\%$, responding to the two ports immediately to the left and right of center was $44.8 \pm 7.7\%$, and responding to the two outside ports was $34.0 \pm 5.2\%$. Premature responses to CRF ($36.1 \pm 5.0\%$) and PRF ($42.7 \pm 5.7\%$) ports did not differ significantly, $p > 0.200$. This pattern of responding suggests the rats tended to orient toward the center port prior to cue delivery, and that anticipatory responses were more related to that orientation than to the tendencies to respond to those ports when they were illuminated (recall that responding to the center port after it was illuminated was lower than responding to any of the other illuminated cues).

Despite the lack of a penalty for perseverative responding (responding to a reinforced cue beyond the single response needed to deliver food) in our study (beyond the self-produced delay of reinforcement such perseveration would produce), these responses were observed on fewer than 2% of the test trials and were unaffected by cue duration, $F < 1$, $p > 0.657$.

Discussion

In Experiment 1, partially-reinforced cues were less likely to control correct responses and more likely to elicit errors than consistently-reinforced cues. Furthermore, correct responding to partially-reinforced cues was more sensitive to disruption by cue duration challenges than responding to consistently reinforced cues. One interpretation of this latter finding is that the rats allocated less attention to the PRF cues than to the CRF cues within the 5-cue array and hence performance to PRF cues was more disrupted by the further demands placed on attentional processing by shortening their duration. Thus, unlike in tasks that assess the acquisition of new learning (e.g., Collins & Pearce, 1985; Collins et al., 1983; Kaye & Pearce, 1984), partially-reinforced cues control less attention than consistently-reinforced in the task studied here.

It is important to note that Pearce and Hall (1980) distinguished between “controlled” attentional processing, which influences cue associability and the acquisition of new learning, and “automatic” attentional processing, which reflects the ability of cues to control already-acquired responding. Within this notion of controlled attention, a cue's associability (a learning rate parameter reflecting the ease with which a cue may enter into new associations on subsequent trials) is proportional to the unsigned prediction error generated in the presence of those cues on preceding trials. Thus, as a consistently-reinforced cue becomes a more accurate predictor of reinforcement, its associability will decline. At the same time, the associability of a cue governs only the rate of learning about it, and not its ability to capture automatic attention for the purposes of directed action. Thus, even as the associability of a CRF cue declines, its ability to control action will be maintained (Hall & Pearce, 1979). By contrast, inconsistent reinforcement of a cue (PRF) or the surprising omission of reinforcement enhances that cue's associability (Hall & Pearce, 1979; Kaye & Pearce, 1984) but may still reduce responding controlled by that cue, as in the present experiment (see also Holland, 2005).

Experiment 2

In Experiment 2, we used the rats from Experiment 1 to examine the effects of disconnecting CeA from SI/nBM on the control of attention by the port cues in the same 5CSRT task as was used in Experiment 1. If CeA works together with SI/nBM in maintaining attention to cues under conditions of attentional challenge, then rats with lesions that disconnect those two brain regions should be more susceptible to the cue duration challenges than rats with control lesions.

All rats received unilateral lesions of CeA and SI/nBM. Half of the rats received these lesions in the same hemisphere (Group Ipsi) and the other half received them in opposite hemispheres (Group Contra). Because projections of CeA to SI/nBM are predominantly ipsilateral, contralateral lesions of CeA and SI/nBM prevent communication between these two regions. Importantly, these lesions spare functions served by each region unilaterally, except for those that require CeA-SI/nBM communication. By contrast, ipsilateral lesions destroy the same amount of tissue in each region as the contralateral lesions, but leave communication between CeA and SI/nBM intact in one hemisphere. Thus, the ipsilaterally-lesioned rats serve as appropriate controls for assessing the effects of CeA-SI/nBM disconnection in the contralaterally-lesioned rats.

Methods and materials

Subjects and apparatus—The subjects and apparatus were the same as in Experiment 1.

Surgery—Rats were anesthetized with isoflurane gas (Abbott Laboratories, North Chicago, IL), and stereotaxic surgery was conducted under aseptic conditions. All rats received unilateral lesions of CeA and SI/nBM. Eight rats received these lesions in the same hemisphere (Group Ipsi) and eight received them in opposite hemispheres (Group Contra). CeA lesions were made using 0.25 μ l of 10 μ g/ μ l ibotenic acid (Biosearch Technologies, Novato, CA) in PBS solution, infused with a Hamilton 2.0 μ l syringe over a 6-min period, at stereotaxic coordinates 2.4 mm posterior to bregma and 4.35 mm from the midline, with infusions at a depth of 7.9 mm from the skull surface. SI/nBM lesions were made using 0.20 μ l of 192IgG-saporin (Chemicon, Temecula, CA; diluted to 0.25 μ g/ μ l in Dulbecco's saline), infused into each of two sites within SI/nBM. The coordinates for these sites were 0.75 mm posterior to bregma, at 2.3 mm and 3.3 mm lateral from the midline, at depths of 7.8 mm and 8.1 mm from the skull surface, respectively.

Training procedures—After recovery from surgery, the rats received six CRF/PRF retraining sessions, followed by another series of attentional challenge sessions. These sessions were identical to the last six training sessions and attentional challenge sessions of Experiment 1.

Histology—After completion of behavioral testing, the rats were anaesthetized and perfused with 0.9 % saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Brains were removed, post-fixed and cryoprotected overnight in 4% paraformaldehyde in 0.1 M PB containing 12% sucrose, frozen with powdered dry ice, and stored at -80°C . Sections (30- μ m) were taken from each brain on a freezing microtome and alternate sections were mounted on slides. One series was Nissl-stained and the other was stained for acetylcholinesterase (AChE), using a standard protocol (Bucci et al., 1998) adapted from Karnovsky & Roots (1964). Lesions of CeA were evaluated by inspection of the Nissl-stained sections. Cholinergic lesions targeting SI/NMB were evaluated by comparing the optical densities of AChE-staining in the hemispheres ipsilateral and contralateral to the SI/nBM lesions, at four anterior-posterior planes. For each rat, we obtained the optical densities for identically-sized regions of neocortex, hippocampus, and striatum. For each region, a ratio that contrasted the optical density in that

region contralateral to the SI/nBM lesion with the density in the same region ipsilateral to the lesion was calculated.

Results

Histology—Thirteen rats were judged as having acceptable lesions of both CeA and SI/nBM (7 were in Group Contra and 6 in Group Ipsi). Acceptable CeA lesions averaged 65% damage to medial CeA and 50% damage to CeA overall. CeA lesions were rejected ($n = 2$) if there was less than 40% damage to the medial portion of CeA. Figure 2 shows photomicrographs of typical intact and lesioned CeAs. CEA lesions in Groups Contra and Ipsi were comparable. Accepted SI/nBM lesions showed cortical contralateral/ipsilateral optical density ratios > 1.6 (mean = 1.81 ± 0.31), and striatal and hippocampal optical density ratios > 0.8 and < 1.2 (mean = 1.04 ± 0.03 and 0.99 ± 0.05). One SI/nBM lesion was rejected; the cortical optical density ratio in that brain was 0.92. The density ratios were comparable in Groups Contra (1.83) and Ipsi (1.79). We did not characterize cell loss in SI/nBM itself. However, it is notable that after using the same lesion procedures and batch of 192IgG-saporin in another experiment, Holland and Gallagher (2006) performed choline acetyl transferase (ChAT) and parvalbumin immunostaining procedures on SI/nBM tissue. They found moderate (but incomplete) loss of ChAT-positive neurons confined mostly to SI/nBM, with no loss of such neurons in the vertical limb of the diagonal band, and only sporadic loss of neurons in the horizontal limb of the diagonal band. Furthermore, there were no differences in parvalbumin immunostaining between the lesioned and unlesioned hemisphere throughout this region, indicating that the IgG192-saporin lesions were highly specific.

Behavior—After some initial disruption in both groups, the rats recovered their original (Experiment 1) levels of responding. Figure 3 shows the primary data of Experiment 2, the results of the baseline and cue duration challenge sessions. Performance of the ipsilaterally-lesioned rats (Figures 3A and 3B) was similar to performance in Experiment 1, prior to surgery. These rats showed greater correct responding and fewer errors to CRF than to PRF cues, disruptive effects of cue duration challenges on performance (fewer correct responses and more errors), and a greater disruptive effect of cue duration challenge on responding to PRF cues than to CRF cues. Thus, when compared to presurgical performance, the ipsilateral lesion had no discernible effect on performance.

In baseline sessions, rats with contralateral disconnection lesions (Figures 3C and 3D) showed responding to CRF cues that was similar to that of the ipsilateral control rats, and hints of slightly greater correct responding to PRF cues than found in the ipsilateral control rats. By contrast, cue duration challenges, thought to reveal differences in attention, disrupted correct responding to both CRF and PRF cues more in contralaterally-lesioned rats than in ipsilateral control rats. This finding shows that a circuit including both CeA and SI/nBM is important for the maintenance of attention to briefly-presented cues in this task, regardless of training contingencies (CRF or PRF). Finally, errors (Figures 3B and D) were not affected by lesion condition.

As in Experiment 1, responding to the EXT cue showed no evidence of stimulus control. Furthermore, responding to that cue was not affected by lesion condition, $F_s < 1$, $p_s > 0.357$. Thus, we do not consider this responding further.

Initial ANOVAs showed that the performances of the two surgical groups were identical prior to surgery. ANOVAs of the data from Experiment 1 (shown in Figure 1) that included lesion assignment condition as a factor showed no significant main effect of that variable, nor any interaction of that variable with reinforcement contingency or cue duration, $F_s < 1$, $p_s > 0.652$. Furthermore, ANOVAs that evaluated changes between pre- and post-surgical tests (Experiments 1 and 2) led to the same conclusions as ANOVAs of Experiment 2's data alone.

A lesion group (contralateral or ipsilateral) X reinforcement contingency (CRF or PRF) X cue duration (500 ms, 250 ms, or 100 ms) ANOVA for correct responding showed significant main effects of contingency, $F(1, 11) = 13.02$, $p = 0.004$, and cue duration, $F(2, 22) = 84.03$, $p < 0.001$. The interaction of those two effects was marginally significant, $F(2, 22) = 3.33$, $p = 0.054$, and a planned contrast of CRF and PRF responding on baseline versus challenge sessions, like that performed in Experiment 1, was significant, $F(1, 11) = 4.86$, $p = 0.0497$. Thus, as in Experiment 1, responding to the PRF cues was especially sensitive to the cue duration challenge. Most important, cue duration interacted significantly with lesion treatment, $F(2, 22) = 10.57$, $p < 0.001$. However, neither the lesion X contingency interaction, $F < 1$, $p = 0.463$, nor the three-way interaction, $F < 1$, $p = 0.545$, was significant.

As in Experiment 1, comparable ANOVAs for errors showed significant effects of contingency, $F(1, 11) = 5.07$, $p = 0.046$, and cue duration, $F(2, 22) = 11.35$, $p < 0.001$. Unlike with correct responses, for errors, neither the main effect of lesion nor any of its interactions, $F_s < 0.326$, $p_s > 0.286$, was significant. Furthermore, comparisons like those described for correct responses were not significant, $F_s < 1$, $p_s > 0.950$.

Part of the basis for attributing the impairments in correct responding produced by CeA-SI/nBM disconnection to attention was the lack of a detrimental effect of that disconnection on baseline performance, in the absence of cue-duration challenges. In that context, we were concerned with our observation of a slight enhancement of baseline PRF performance in contralaterally-lesioned rats. We conducted post-hoc Tukey HSD comparisons of correct responding on 500-ms (baseline) and 100-ms (challenge) tests on CRF and PRF trials. The rats in group Ipsi showed significantly ($p_s < 0.050$) more correct responding on 100-ms trials than the rats in Group Contra on both CRF and PRF trials, but the apparently greater responding of Group Contra on baseline PRF trials was not significant, ($p = 0.607$). Nevertheless, it is worth noting that Maddux and Holland (2004) observed a similar enhancement after bilateral infusions of 192IgG-saporin into the medial prefrontal cortex, which destroy a population of SI/nBM cholinergic neurons that project to that cortical region. We have no ready account for that observation.

Other response measures—As in Experiment 1, both omissions and perseverative responses were rare (less than 5% and 2% of trials, respectively), and neither was affected by lesion, $F_s < 1$, $p_s > 0.506$. Also as in Experiment 1, premature responses showed a significant spatial bias, favoring the center port. A lesion X spatial position ANOVA revealed a significant main effect of spatial position, $F(2, 22) = 7.67$, $p = 0.003$, and no effect of lesion, $F(1, 11) < 1$, $p = 0.916$. The lesion X position interaction was marginally significant, $F(2, 22) = 3.15$, $p = 0.063$, reflecting a tendency for contralaterally-lesioned rats to show a shallower spatial gradient. Premature responding to the center, immediately-adjacent, and end ports was $56.9 \pm 9.2\%$, $48.6 \pm 15.0\%$, and $25.0 \pm 7.5\%$, respectively, in the ipsilaterally-lesioned rats, and $51.2 \pm 12.6\%$, $41.6 \pm 8.1\%$, and $41.7 \pm 8.1\%$ in the contralaterally-lesioned rats.

Discussion

Although it is difficult to compare the results of Experiment 2 directly with those of studies from other laboratories, which used different training and lesion procedures, it is notable that our general conclusion that CeA-SI/nBM disconnection interferes with attention for performance in this task is consistent with previous findings of the effects of bilateral nBM lesions on 5CSRT performance. For example, Muir et al. (1994) found that shortening cue durations impaired the performance of rats with AMPA lesions of nBM more than that challenge hurt the performance of control rats. Similarly, McGaughy et al. (2002, Exp. 2) found that bilateral infusion of relatively low levels ($0.075 \mu\text{g/hemisphere}$) of 192IgG-saporin into nBM impaired attention in the 5CSRT, as indicated by reduced accuracy when the density

of trials was increased, and Waite et al. (1999) found that intraventricular administration of 192IgG-saporin exacerbated the detrimental effects of a number of manipulations thought to affect attention in the 5CSRT task, including reductions in event rate, introduction of distractors, and increasing the array size (number of cue ports).

By contrast, neither Waite et al.'s (1999) nor McGaughey et al.'s (2002) lesioned rats showed performance deficits when cue duration was shortened, the manipulation we used to assess attention for action. Similarly, Chudasama et al. (2004) found no effect on response accuracy when cue duration was reduced from 1 s to 250 ms (although they did see a threefold increase, relative to controls, in the number of trials on which lesioned rats failed to respond, which might also indicate impairment in detecting or attending to those cues). Thus, the role of SI/nBM itself in this task is still unclear.

It is also worth noting that some investigators have reported more pervasive effects of SI/nBM lesions than we observed here. When McGaughey et al. (2002, Exp. 1) made lesions with 0.225 µg of 192IgG-saporin (more than double our dose), the lesions had profound effects on baseline 500-ms cue performance, which emerged over 5-10 sessions of post-surgical retraining. These latter effects, which were attributed to deficits in attention because they were alleviated by lengthening the cue durations, included decreases in accuracy, increases in trials with no responses, increased response latencies, and increases in both perseverative and premature responses. We found none of these effects on baseline 500-ms cue duration performance, and in our study performance improved from non-specific (i.e., found in both lesion groups) post-surgical deficits (not shown) over the course of 9 retraining sessions, rather than showing declines as in McGaughey et al.'s (2002) study.

Although it might be argued that the more limited effects of CeA-SI/nBM disconnection lesions in our study reflects, a specific, more limited role for CeA in modulating SI/nBM function, they may also be attributable to variations in the size of the SI/nBM lesions across studies. For example, it is likely that the extent of the lesions found with McGaughey et al.'s (2002) high toxin dose was much greater than the extent of our SI/nBM lesions. Their lesions included nearly complete loss of ChAT-positive neurons in SI/nBM as well as losses in both the horizontal and vertical limbs of the diagonal band. As noted earlier, although we did not examine ChAT in the present study, using the same lesion procedures and batch of 192IgG-saporin, Holland and Gallagher (2006) found no loss of ChAT-positive neurons in the vertical limb of the diagonal band, only sporadic loss of neurons in the horizontal limb, and moderate but incomplete loss in SI/nBM.

At the same time, many of these behavioral differences may be related to our 5CSRT training procedures, which do not involve penalties for inappropriate responses (see methods and results sections). Robbins (2002) has persuasively argued that these additional contingencies provide the opportunity for a more detailed analysis of a number of subclasses of attentional deficits. Our contingencies result in considerably higher rates of premature and error responses, and lower rate of omissions than observed with procedures that include trial cancellation for inappropriate responses. Thus, it is perhaps not surprising that these measures failed to show significant sensitivity to lesion manipulations in our study. Nevertheless, it is not clear how the lack of penalties for errors, premature and perseverative responses would make our procedure more likely to reveal lesion effects on cue duration challenges.

General Discussion

In both intact rats (Experiment 1) and rats with ipsilateral control lesions of CeA and SI/nBM (Experiment 2), partially-reinforced cues were less likely to control correct responses and more likely to elicit errors than consistently-reinforced cues. Furthermore, correct responding to

partially-reinforced cues was more sensitive to disruption by cue duration challenges than responding to consistently reinforced cues. One interpretation of this latter finding is that the rats allocated less attention to the PRF cues than to the CRF cues within the 5-cue array and hence performance to PRF cues was more disrupted by the further demands placed on attentional processing by shortening their duration.

Disconnection of CeA and SI/nBM did not significantly affect baseline performance, but impaired performance when cue duration was shortened in the attentional challenge sessions, for both CRF and PRF cues. Holland et al. (2000), using a 3-choice variant of this task, found that rats with bilateral lesions of CeA also showed substantial impairments in responding in challenge tests, but not under baseline conditions. Thus, the present results extend Holland et al.'s (2000) findings by showing that CeA's influence on attention in the control of current action in this task is mediated at least in part by its communication with cholinergic neurons in SI/nBM.

Our observation that CeA-SI/nBM disconnection lesions impair attention in the control of current action in the 5CSRT task parallels our earlier findings that such lesions, made prior to training, also impair surprise-induced enhancements in CS associability (Han et al., 1999). At first glance, these two sets of results taken together suggest that CeA and SI/nBM modulate a unitary cholinergic attention system that acts on early-stage sensory processing, influencing both automatic and controlled attention (within Pearce & Hall's, 1980, framework), or indeed any function controlled by a given stimulus. This view is reminiscent of early reports that intact CeA function is required for the synchronization of cortical EEG by basal forebrain structures (Kapp et al., 1992).

However, other data suggest separation of learning-based and action-based attention systems at some levels. First, the functions of this circuit may differ somewhat in these two tasks, and in attention in action and in new learning more generally. Note that in Experiment 2, communication between CeA and SI/nBM was important for the performance of previously well-learned responses under conditions of attentional challenge. Thus, this communication was important for the expression of this aspect of attentional processing. By contrast, CeA-SI/nBM communication may not be critical to the expression of enhanced CS associability, once those enhancements are accomplished. Holland and Gallagher (2006) found that as long as CeA function was intact when surprise was induced, interference with CeA function later, when the surprise-enhanced associability was assessed in a phase of new learning, had no effect. Thus, unlike with the expression of attention in action in Experiment 2, CeA-SI/nBM communication was apparently not necessary at the time of the expression of previously-enhanced CS associability in new learning.

Second, frequent observations that procedural manipulations that enhance controlled attention impair or have no effect on automatic attention make a unitary, early-processing account seem less plausible. For example, in the present study, a stimulus validity manipulation that typically enhances associability of CSs in tests of new learning (e.g., Kaye & Pearce, 1984) reduced the ability of a previously-trained cue to control responding in the face of reduced cue duration. If partial reinforcement of a cue enhances its associability by facilitating sensory processing of that cue, then that manipulation should enhance any measure of attention. Instead, the opposite effect was observed in the present experiments.

Thus, we suggest that the CeA-SI/nBM system modulates the action of two different attentional networks, one responsible for enhancement of controlled attention for purposes of new learning and one responsible for automatic attention to cues for the purpose of current action. In support of this suggestion, Maddux et al. (2005) found that disruption of the cholinergic innervation of the posterior parietal cortex (PPC) by SI/nBM interfered with surprise-induced

enhancements of controlled attention for new learning (see also Bucci et al., 1998) but not with automatic attention for action, as assessed in a 5CSRT task like that used in the present study. By contrast, disruption of the cholinergic input of the medial prefrontal cortex by SI/nBM interfered with attention for current action (see also Gill et al., 2000) but not surprise-induced attention for new learning. Thus, these attentional systems may act more separately and directly on mechanisms of learning and action, respectively, rather than modulating early-stage sensory processing. This dissociation makes good adaptive sense: current action should be controlled by the best predictors of the future, but attentional resources for new learning should be allocated to cues whose consequences have not yet been well-determined. Further investigation of the role of prediction and prediction error (“surprise”) in the modulation of distinct cortical circuits for these aspects of attention in learning and action should prove valuable for understanding basic mechanisms of adaptation to changing environments.

Acknowledgements

I thank Erin Kerfoot and Vanessa McKenna for their assistance. Supported by NIH MH53667.

References

- Bucci DJ, Holland PC, Gallagher PC. Removal of cholinergic input to rat posterior parietal cortex disrupts incremental processing of conditioned stimuli. *Journal of Neuroscience* 1998;18:8038–8046. [PubMed: 9742170]
- Chiba AA, Bucci DJ, Holland PC, Gallagher M. Basal forebrain cholinergic lesions disrupt increments but not decrements in conditioned stimulus processing. *Journal of Neuroscience* 1995;15:7315–7322. [PubMed: 7472485]
- Chiba AA, Bushnell PJ, Oshiro WM, Gallagher M. Selective removal of cholinergic neurons in the basal forebrain alters cued target detection in rats. *NeuroReport* 1999;10:1–5. [PubMed: 10094123]
- Chudasama Y, Dalley JW, Falguni N, Bouger P, Robbins TW. Cholinergic modulation of visual attention and working memory: Dissociable effects of basal forebrain 192-IgG-saporin lesions and intraprefrontal infusions of scopolamine. *Learning and Memory* 2004;11:78–86. [PubMed: 14747520]
- Collins L, Pearce JM. Predictive accuracy and the effects of partial reinforcement on serial autoshaping. *Journal of Experimental Psychology Animal Behavior Processes* 1985;11:548–564.
- Collins L, Young DB, Davies K, Pearce JM. The influence of partial reinforcement on serial autoshaping with pigeons. *Quarterly Journal of Experimental Psychology* 1983;35B:275–290. [PubMed: 6686883]
- Everitt BJ, Robbins TW. Central cholinergic systems and cognition. *Annual Review of Psychology* 1997;48:649–684.
- Gallagher, M.; Holland, P. Understanding the function of the central nucleus: Is simple conditioning enough?. In: Aggleton, JP., editor. *The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction*. New York: Wiley-Liss; 1992. p. 307–321.
- Gill TM, Sarter M, Givens B. Sustained visual attention performance-associated prefrontal neuronal activity evidence for cholinergic modulation. *Journal of Neuroscience* 2000;20:4745–4757. [PubMed: 10844044]
- Hall G, Pearce JM. Latent inhibition of a CS during CS-US pairings. *Journal of Experimental Psychology: Animal Behavior Processes* 1979;5:31–42. [PubMed: 528877]
- Han JS, Holland PC, Gallagher M. Disconnection of the amygdala central nucleus and substantia innominata/nucleus basalis disrupts increments in conditioned stimulus processing in rats. *Behavioral Neuroscience* 1999;113:143–151. [PubMed: 10197914]
- Holland PC. Brain mechanisms for changes in processing of conditioned stimuli in Pavlovian conditioning: Implications for behavior theory. *Animal Learning & Behavior* 1997;25:373–399.
- Holland PC. Amount of training effects in representation-mediated food aversion learning: No evidence for a role for associability changes. *Learning & Behavior* 2005;33:464–478. [PubMed: 16573217]
- Holland PC, Han JS, Gallagher M. Lesions of the Amygdala central nucleus alter performance in a selective attention task. *Journal of Neuroscience* 2000;20:6701–6706. [PubMed: 10964975]

- Holland PC, Gallagher M. Amygdala central nucleus lesions disrupt increments, but not decrements, in CS processing. *Behavioral Neuroscience* 1993;107:246–253. [PubMed: 8484890]
- Holland PC, Gallagher M. Amygdala circuitry in attentional and representational processes. *Trends in Cognitive Science* 1999;3:65–73.
- Holland PC, Gallagher M. Different roles for amygdala central nucleus and substantia innominata in the surprise-induced enhancement of learning. *Journal of Neuroscience* 2006;26:3791–3797. [PubMed: 16597732]
- Kapp, BS.; Whalen, PJ.; Supple, WF.; Pascoe, JP. Amygdaloid contributions to conditioned arousal and sensory information processing. In: Aggleton, J., editor. *The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction*. Chichester: Wiley-Liss; 1992. p. 229-254.
- Karnovsky MJ, Roots L. A “direct-coloring” thiocholine method for cholinesterases. *Journal of Histochemistry and Cytochemistry* 1964;12:219–221. [PubMed: 14187330]
- Kaye H, Pearce JM. The strength of the orienting response during Pavlovian conditioning. *Journal of Experimental Psychology: Animal Behavior Processes* 1984;10:90–109. [PubMed: 6707583]
- Mackintosh NJ. A theory of attention: Variations in the associability of stimuli with reinforcement. *Psychological Review* 1975;82:276–298.
- Maddux JM, Chatterjee S, Kerfoot EC, Holland PC. Distinct roles for amygdala central nucleus, medial prefrontal cortex, and posterior parietal cortex in attention for learning and action. *Society for Neuroscience* 2005;Abstract 411.16
- Maddux JM, Holland PC. Lesions of medial prefrontal cortex interfere with rats' sensitivity to the predictive value of cues in a 5-choice serial reaction time task. *Society for Neuroscience* 2004;Abstract 322.10
- McGaughy J, Dalley JW, Morrison CH, Everitt BJ, Robbins TW. 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. *Journal of Neuroscience* 2002;22:1905–1913. [PubMed: 11880520]
- McGaughy J, Kaiser T, Sarter M. Behavioral vigilance following infusions of 192 IgG-saporin into the basal forebrain: selectivity of the behavioral impairment and relation to cortical AChE-positive fiber density. *Behavioral Neuroscience* 1996;110:247–265. [PubMed: 8731052]
- McGaughy J, Sarter M. Behavioral vigilance in rats: task validation and effects of age, amphetamine and benzodiazepine receptor ligands. *Psychopharmacology* 1995;117:340–357. [PubMed: 7770610]
- Muir JL, Everitt BJ, Robbins TW. AMPA-induced excitotoxic lesions of the basal forebrain: A significant role for the cortical cholinergic system in attentional function. *Journal of Neuroscience* 1994;14:2313–2326. [PubMed: 7512637]
- Pearce JM, Hall G. A model for Pavlovian learning: variations in the effectiveness of conditioned but not of unconditioned stimuli. *Psychological Review* 1980;87:532–552. [PubMed: 7443916]
- Robbins TW. The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. *Psychopharmacology* 2002;163:362–380. [PubMed: 12373437]
- Sarter M, Bruno JP. Cognitive functions of cortical acetylcholine: toward a unifying hypothesis. *Brain Research Reviews* 1997;23:28–46. [PubMed: 9063585]
- Voytko ML. Cognitive functions of the basal forebrain cholinergic system in monkeys: memory or attention. *Behavioral Brain Research* 1996;75:13–25.
- Waite JJ, Wardlow ML, Power AE. Deficit in selective and divided attention associated with cholinergic basal forebrain immunotoxic lesion produced by 192-saporin; motoric/sensory deficit associated with pukinje cell immunotoxic lesion produced by OX7-saporin. *Neurobiology of Learning and Memory* 1999;71:325–352. [PubMed: 10196110]

Figure 1.

Performance in baseline (500-ms) and cue duration challenge (250-ms and 100-ms) sessions in Experiment 1. Panel A shows the percentage of trials with at least one correct response and panel B shows the percentage of trials with at least one error response. CRF = consistent reinforcement, EXT = extinction, PRF = partial reinforcement.

Figure 2.

Photomicrograph of intact CeA (A) and lesioned CeA (B); the lesion of medial CeA is outlined. BLA = basolateral amygdala, CeA = central nucleus of amygdala, IA = intercalated nuclei of amygdala.

Figure 3. Performance in baseline (500-ms) and cue duration challenge (250-ms and 100-ms) sessions in Experiment 2. Panels A and C show the percentages of trials with at least one correct response and panels B and D show the percentages of trials with at least one error response. Panels A and B show responding of ipsilaterally-lesioned control rats, and panels C and D show the performance of rats with contralateral lesions that disconnected CeA and SI/nBM. CeA = central nucleus of amygdala, contra = contralateral lesions, CRF = consistent reinforcement, EXT = extinction, ipsi = ipsilateral lesions, PRF = partial reinforcement.

Table 1

Distribution of errors across ports in Experiment 1

Cue type	correct	Response type CRF port error	PRF port error	EXT port error
500ms baseline				
CRF	93.8 ± 1.4	33.6 ± 2.2	25.2 ± 2.2	23.5 ± 3.4
PRF	83.3 ± 2.5	33.6 ± 2.8	30.8 ± 5.4	32.8 ± 3.5
EXT	74.4 ± 5.0	72.6 ± 5.1	68.6 ± 7.6	----
250 ms challenge				
CRF	91.7 ± 0.6	52.0 ± 4.5	38.2 ± 6.8	46.1 ± 6.6
PRF	74.4 ± 4.2	53.1 ± 6.6	44.7 ± 5.8	49.9 ± 5.9
EXT	69.8 ± 7.8	63.6 ± 5.0	56.9 ± 5.5	----
100 ms challenge				
CRF	77.7 ± 4.1	46.5 ± 5.7	51.2 ± 5.3	55.0 ± 8.9
PRF	57.7 ± 3.3	48.2 ± 6.4	49.9 ± 6.0	51.0 ± 5.7
EXT	73.5 ± 7.9	71.9 ± 8.0	63.5 ± 7.5	----

Note. Entries are percentage (mean ± sem) of CRF, PRF, and EXT trials on which at least one of each type of response occurred, in sessions in which the cue durations were 500 ms, 250 ms or 100 ms. Entries for CRF port errors on CRF trials and PRF port errors on CRF trials are averages of these percentages for the two possible ways of making such an error. CRF = Consistent reinforcement, EXT = extinction, PRF = partial reinforcement.