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Habituation and Retention of the Head-Shake Response: Lack of Impairment by Nucleus Basalis Magnocellularis Lesions

CARL P. J. DOKLA,[1] SEAN C. PARKER

Department of Psychology, Fairfield University, Fairfield, CT 06430

AND

LEON J. THAL[2]

Neurology Service, San Diego VA Medical Center
and Department of Neurosciences, University of California, San Diego
San Diego, CA 92161

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DOKLA, C. P. J., S. C. PARKER AND L. J. THAL. *Habituation and retention of the head-shake response: Lack of impairment by nucleus basalis magnocellularis lesions.* PHARMACOL BIOCHEM BEHAV **35**(1) 151–155, 1990.—Bilateral ibotenic acid lesions of the nucleus basalis magnocellularis (NBM) were examined on the habituation and retention of the head-shake response (HSR). HSR, a rapid, stereotyped rotation of the head about a front-to-rear axis, was elicited by a stream of mildly pressurized air directed at the ear in Fischer 344 rats. HSR training consisted of 40 stimulated trials followed by a 30-min retention test of 20 stimulated trials. Stimulus duration was 15 sec per trial with a 15-sec intertrial interval. Frontal cortex choline acetyltransferase was reduced by 22% in the NBM-lesioned group compared to the controls. The NBM-lesioned rats and the controls were not significantly different on either frequency or latency measures of HSR habituation or retention. The results do not support a role for cortical cholinergic mediation of the HSR.

Head-shake response Habituation Nucleus basalis magnocellularis lesions Cortical choline acetyltransferase Retention Rat

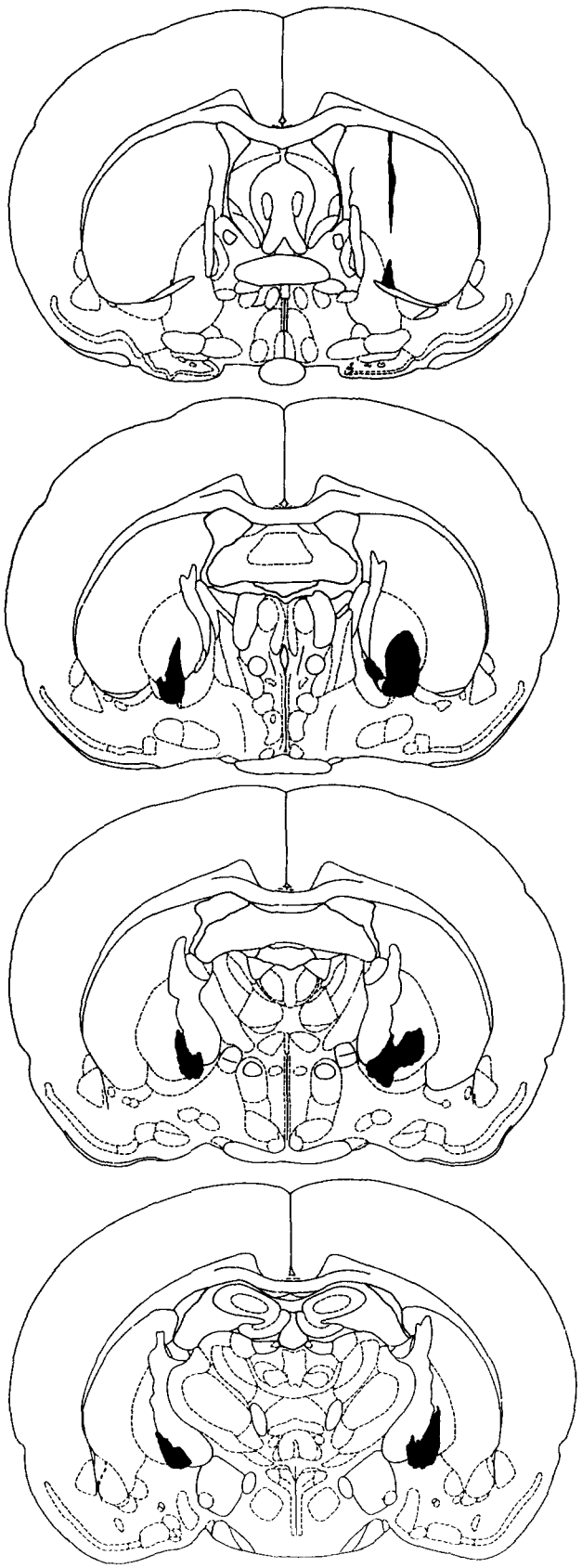
HABITUATION may be defined as response decrement to an initially novel stimulus resulting from repeated elicitation (7, 13, 20). Two major neural systems models of habituation have been proposed to explain habituation. In the "dual-process theory," Groves and Thompson (5) propose that habituation is the result of polysynaptic depression in the stimulus-response pathway and that it is a useful model for the study of short-term habituatory decrements (13). In a second model proposed by Sokolov (19) cortical representation of the habituatory stimulus develops which inhibits the reticular activating system; response strength is determined by the degree of dissimilarity between the cortical representation and the stimulus input.

Williams, Hamilton and Carlton (22) divided habituatory responses into two classes: those that are *emitted* by the organism, such as exploratory behavior, and those that are *elicited* from an organism, such as startle. Since the muscarinic antagonist, scopolamine, disrupted habituation of exploration (head-poke response), but not startle, it was suggested that the cholinergic system may be important in habituation of emitted types of responses. O'Keefe and Nadel (13) suggested, consistent with Williams *et al.* (22), that the septo-hippocampal system, a principal cholinergic system, is critical to locale function, i.e., spatial mapping, and, therefore, important for habituation of exploratory behavior, but not responses which can be maintained by taxon functions (nonspatial guidance and orientation), such as startle, arrest, and orienting.

The present investigation examined the importance of the cortical cholinergic system to habituation of an elicited behavior, the head-shake response (HSR) (1). HSR is a rapid (ca. 160 msec), stereotyped rotation of the head about its anterior-posterior axis which in the rat may be elicited as an unconditioned response by a stream of mildly pressurized air directed at the pinna of the ear (1). HSR occurs in a discrete, all-or-none fashion which facilitates unequivocal rating and the response has been parametrically evaluated in detail (10). Habituation of HSR occurs during

[1]Present address: Department of Psychology, Saint Anselm College, Manchester, NH 03102.

[2]Requests for reprints should be addressed to Leon J. Thal, M.D., Neurology Service (127), VA Medical Center, 3350 La Jolla Village Drive, San Diego, CA 92161.



acquisition. Habituation disappears completely at 24 hours and recovery of approximately 50% is typical at 30 minutes posthabituation (10).

In the present study, bilateral ibotenic acid (IBO) lesions of the nucleus basalis magnocellularis (NBM) which can impair cortical cholinergic function and behavior and deplete choline acetyltransferase (CAT) [see reviews by Collerton (2), and Smith (18)] were evaluated on habituation and retention of the HSR in rats.

METHOD

Subjects

Sixteen male Fischer 344 rats with a mean weight of approximately 346 g were used. All rats were housed individually in stainless steel wire mesh cages under a 12:12 light/dark cycle (light from 0900 to 2100 hr) and permitted food and water ad lib.

Surgery

Ten randomly selected rats received bilateral stereotaxic NBM lesions in two stages separated by 3 weeks between surgeries. Rats were anesthetized using a mixture of 7.5 ml of Ketamine hydrochloride (Ketaset, 100 mg/ml), 1.9 ml of Xylazine (Rompun, 20 mg/ml), 0.75 ml of acepromazine maleate (10 mg/ml), and 20 ml of 0.9% saline; the drug was administered intramuscularly at a dose of 0.25 ml/0.1 kg bodyweight. IBO (Sigma), 6 μg, was dissolved in 1 μl of distilled water and infused in the NBM over 20 min (0.05 μl at 1-min intervals) followed by an additional 2 min in which the syringe needle (0.64 mm o.d.; 5 μl syringe, Hamilton model 7105-N) was left in place to prevent backflow. Lesion coordinates (in mm) were +8.0 AP, ±3.0 ML and +2.9 DV from the interaural line with the skull placed so that bregma and lambda were in the horizontal plane, according to the atlas of Paxinos and Watson (15). Six additional rats received sham operations (CONT), i.e., surgical treatment without intracranial penetration by the syringe needle.

Apparatus

A platform was used to restrict the rat's movements during habituation testing and was constructed using the same materials and dimensions as described in detail by Leibrecht and Askew (8). The platform was mounted atop a steel pipe and was attached to a 4-inch lazy susan-type disk base which allowed the stand to be rotated in either direction.

The test stimulus consisted of constant flow, pressurized air from a Silent Giant aquarium pump (Model 120) and was delivered through a hand-held glass rod (1/16-in. i.d.) which was connected to the air supply by flexible plastic tubing. Air pressure was regulated as per Leibrecht and Askew (10); with the open end of the glass stimulation rod held by a stereotaxic instrument 0.5 mm from the end of an open manometer (1.5 mm i.d.), a 12-cm column of 95% ethanol was displaced 4 cm.

Procedure

Two weeks postoperative recovery followed the second surgery. During the entire second week, all rats were given gentling and handling which also included nonstimulated adaptation exposures to the testing platform.

FIG. 1. Schematic reconstruction of a representative NBM lesion induced by bilateral infusion of IBO. The primary area of neuronal loss and gliosis is depicted by solid black shading [diagrams adapted from (15)].

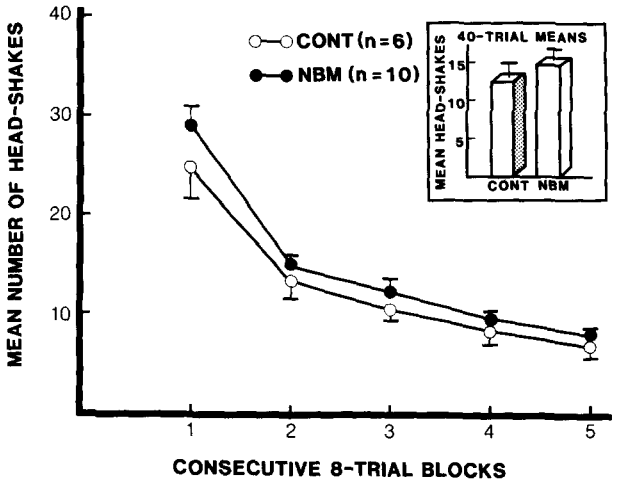


FIG. 2. Mean HSR frequency per group (±SEM, raw scores) for the five 8-trial blocks of habituation acquisition. The insert depicts the 40-trial mean HSR frequency of the NBM-lesioned group and the CONT group.

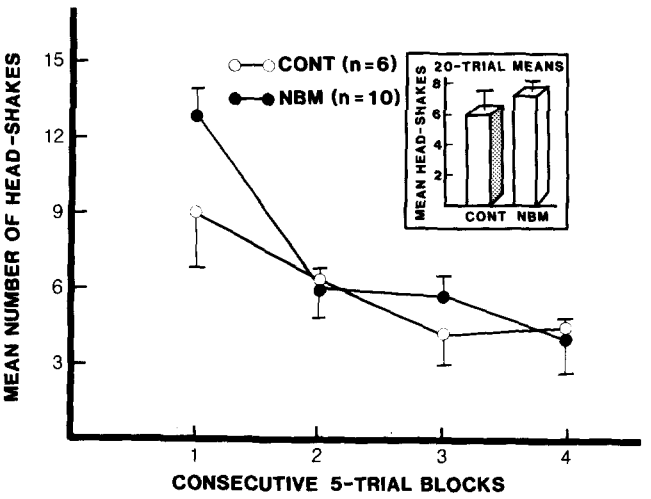


FIG. 4. Mean HSR frequency per group (±SEM, raw scores) for the four 5-trial blocks of habituation retention. The insert depicts the 20-trial mean HSR frequency of the NBM-lesioned group and the CONT group.

HSR was elicited by moving the air stimulus across the center of the left pinna at approximately 3–5 cycles per sec at a distance of approximately ½ in. HSR training consisted of 40 stimulated acquisition trials followed 30 min later by a retention test of 20 stimulated trials. During the 30-min retention interval rats were returned to their home cages (10). Stimulus duration was 15 sec per trial with a 15-sec intertrial interval. Fifteen-sec intervals were timed electronically and acoustic cue signals were delivered by earphone to the experimenters. Testing was conducted between 1000 and 1500 hr and trials were videotaped for subsequent analyses.

Biochemistry and Histology

Rats in the NBM-lesioned group and CONT group were sacrificed by decapitation, the brains were rapidly removed, and tissue samples were excised on a cold glass plate and then frozen on dry ice. Samples were taken from both frontal poles. CAT activities were measured using the radioenzyme technique of Fonnum (4) and corrected for protein by the method of Lowry *et al.* (11). After sampling, the brains were placed in 10% buffered Formalin and later transferred to sucrose-Formalin. The brains were frozen sectioned at 40 μm and sections through the lesion sites were stained with cresyl violet acetate and examined microscopically for cell loss and gliosis.

Statistical Analyses

Group comparisons for the CAT data and retention indexes

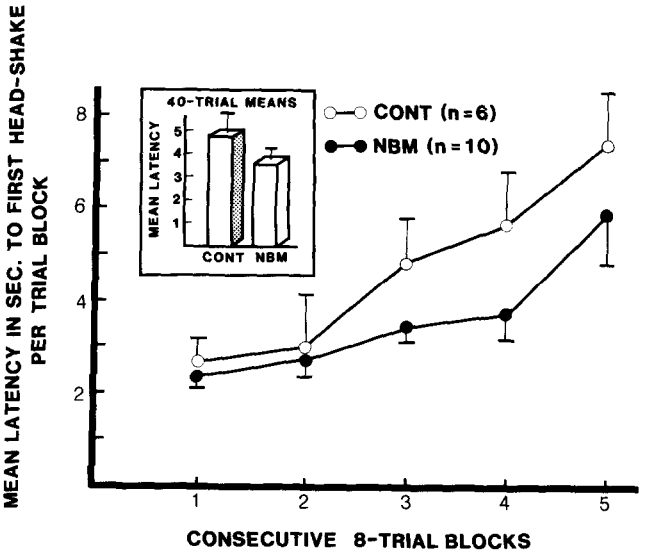


FIG. 3. Mean latency (±SEM, raw scores) to first HSR per trial for the five 8-trial blocks of habituation acquisition. The insert depicts the 40-trial mean HSR latency of the NMB-lesioned group and the CONT group.

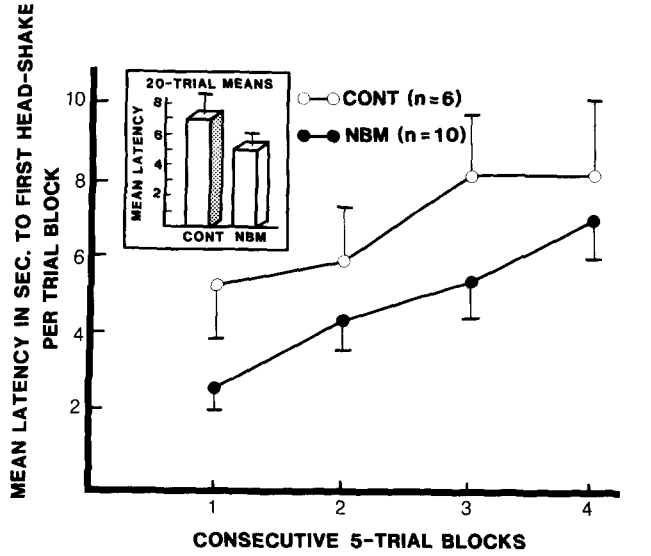


FIG. 5. Mean latency (±SEM, raw scores) to first HSR per trial for the four 5-trial blocks of habituation retention. The insert depicts the 20-trial mean HSR latency for the NBM-lesioned group and the CONT group.

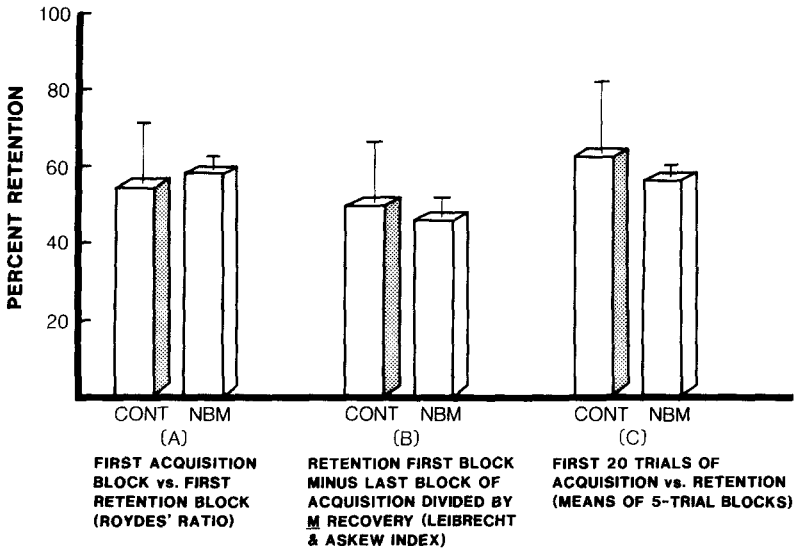


FIG. 6. HSR retention ratios (frequency, raw scores) for the NBM-lesioned group and the CONT group according to three different indexes.

were analyzed by *t*-tests. Behavioral data were analyzed by two-way mixed design (repeated measures on trial blocks) analysis of variance (ANOVA). HSR frequency data were transformed by $\sqrt{X}$, while latency data were transformed by $\log_{10}$ prior to analysis (23).

RESULTS

Biochemical and Histological Results

The NBM-lesioned group had significantly less frontal cortex CAT activity than the CONT group [mean ± SEM: 112.2 ± 2.3 vs. 87.3 ± 2.3 nM ACh/hr/mg protein, $t(13) = 6.67$, $p < 0.001$]. The mean CAT loss in the NBM-lesioned group, compared to CONT, was 22%.

Histological evaluation was conducted on all NBM-lesioned rats. Microscopic examination revealed cell loss and gliosis at the cannula tip with marked gliosis and cell loss in the ventromedial and ventrolateral globus pallidus.

Coronal brain sections from a representative NBM-lesioned rat are depicted in Fig. 1.

Behavioral Results

Figure 2 presents the mean HSR frequency per group for the five 8-trial blocks of habituation acquisition. Consistent with habituation, both the NBM-lesioned and CONT groups progressively decreased HSR frequency over trial blocks, $F(4,56) = 155.61$, $p < 0.001$. However, the groups were not significantly different on either overall habituation, $F(1,14) = 1.94$, or the rate of habituation (treatments × trial blocks interaction), $F(4,56) = 0.35$ (both $ps > 0.05$).

Figure 3 presents the mean latency to first HSR per 15-sec trial for the groups on the five 8-trial blocks of habituation acquisition. Consistent with habituation, both groups progressively increased HSR latency over trial blocks, $F(4,56) = 15.17$, $p < 0.001$. However, the groups were not significantly different on either overall habituation, $F(1,14) = 1.45$, or the rate of habituation, $F(4,56) = 1.06$ (both $ps > 0.05$).

Figure 4 presents the mean HSR frequency per group for the four 5-trial blocks of habituation retention. As per acquisition, both groups progressively decreased HSR frequency over trial blocks, $F(3,42) = 34.02$, $p < 0.001$, but there were no significant group differences in overall habituation, $F(1,14) = 1.35$, or rate of habituation, $F(3,42) = 1.94$ (both $ps > 0.05$).

Figure 5 presents the mean latency to first HSR per 15-sec trial for the groups on the four 5-trial blocks of habituation retention. As per acquisition, all groups progressively increased HSR latency over trial blocks, $F(3,42) = 9.08$, $p < 0.001$, but there were no significant group differences in overall habituation, $F(1,14) = 2.53$, or rate of habituation, $F(3,42) = 1.07$ (both $ps > 0.05$).

Figure 6 presents the mean HSR retention ratios (frequency) for the groups, according to three different retention indexes. In (A) the mean ratio of the first acquisition block to the first retention block is presented [Roydes index; (17)]. In (B) the Leibrecht-Askew index (10) is presented, which is the ratio of the first retention block to the last acquisition block divided by the mean recovery (mean of the last trial block of acquisition minus the mean of the first trial block of acquisition). In (C) the mean ratio of the first 20 trials of acquisition to the 20 trials of retention is presented for each of the groups. There were no significant differences between the groups for any of the retention indexes. Comparable retention-ratio analyses using the latency scores yielded nonsignificant results, as well.

DISCUSSION

The major finding of the present investigation was that IBO lesions of the NBM did not significantly affect the rate or overall habituation of HSR or its retention (30-min recovery). Thus, the present study lends support to Williams *et al.*'s (22) hypothesis that elicited forms of habituation, such as HSR, are not under cholinergic control. Conversely, Williams *et al.*'s (22) hypothesis predicts deficits on habituation of exploratory behavior and locomotor activity after cholinergic loss. Although the effects of NBM lesions on habituation have not been extensively studied, Dubois, Agid, LeMoal and Simon (3) reported reduced locomotor activity habituation in a circular corridor apparatus and no habituation (30-min session) of exploratory behavior (nose poke) in an 8-hole box in NBM-lesioned rats (IBO, mean CAT loss, 32%). Further, Haroutunian, Kanof and Davis (6) reported that NBM-lesioned rats (IBO, mean CAT loss, 21.3%) were impaired on 24-hr retention of locomotor activity habituation. Therefore, there is

confirmatory evidence to support a role for the cortical cholinergic system in emitted forms of habituation, such as exploratory behavior and locomotor activity.

However, there exists the possibility that a critical threshold of cortical CAT loss must be exceeded in order to produce deficient HSR habituation. The decreases in cortical CAT activity obtained in the present study were typically small to moderate relative to other reported studies using IBO [see (18) for a review]. However, inspection of the individual data revealed no relationship between CAT loss and HSR performance, suggesting that the lack of change in HSR following NBM lesion was not secondary to inadequate CAT depletion.

The present study is only the second publication, to our knowledge, to attempt brain-lesion manipulation of HSR. Roydes (17) published the only lesion study using HSR and reported that aspiration lesions of the medial frontal cortex, but not the cingulate gyrus, impaired the rate and retention of HSR habituation in rats. These results contrast with the present findings, since NBM lesions result in partial cholinergic deafferentation of frontal cortex. Although the strain of rats used in the present study (Fischer 344 albinos), differed from that used by Roydes (17) the testing parameters, air-stimulus intensity, and retention interval were the same. Although NBM lesions and frontal cortex lesions can produce somewhat similar behavioral deficits in rats, e.g., Morris water task and radial arm maze (8), lesions of different frontal cortex regions can result in entirely different behavioral effects. For example, head-poke habituation is impaired by medial frontal lesions, but not ventral frontal lesions in the rat (8). These behavioral differences are presumably the result of somewhat selective disruption of frontal cortex efferents and afferents. Medial frontal cortex efferents project to a number of brain stem sites (e.g., pretectum, dorsal raphe, ventral tegmentum, substantia nigra), thalamic sites, and hypothalamic areas (8). In addition, efferents also project to cortical and basal forebrain areas, as well (8). Thus, aspiration lesions may destroy essential connections to subcortical-brain stem sites, which may be part of the essential neural circuitry for HSR. NBM lesions would, of course, spare those fibers of passage and, thus, preserve HSR.

The present results are consistent with findings derived from lesion and drug studies on another elicited form of habituation, the acoustic startle response. Acoustic startle has been reported to be unchanged after treatment with cholinergic agonists: physostigmine and pilocarpine (14); cholinergic antagonists: scopolamine (16,21) and atropine (14); or hippocampal lesions (13). Another type of elicited, reflex-like habituation, the rabbit nictitating membrane response (a subcortical mediated response), has also been reported to be unaffected by scopolamine blockage (12).

The present results do not support frontal cholinergic involvement in HSR habituation. Although Roydes' (17) findings suggest essential frontal cortical involvement in HSR habituation, whatever mechanisms may be involved appear resistant to cholinergic manipulation in this type of elicited behavior.

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