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Transecting the Dorsal Fornix results in Novelty Detection but not Temporal Ordering Deficits in Rats

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Abstract

It has been shown that CA1 subserves temporal ordering processes in the hippocampus. It has also recently been shown that transecting the subcortical outputs of dorsal CA1 via the dorsal fornix results in retrieval deficits similar to those seen after lesions to CA1. The present experiment was designed to evaluate the effects of disrupting CA1 subcortical outputs for the temporal processing of visual objects and for a visual object novelty detection paradigm. The results of the present study suggest that CA1 subcortical efferents are not critically involved in temporal processing of visual objects, but are critically involved in visual object novelty detection. The data also suggest that temporal processing and novelty detection may potentially be subserved by independent mechanisms in CA1.

Keywords

Dorsal Fornix; Temporal Order; Hippocampus; Novelty Detection

Introduction

It has been shown in lesion, pharmacological inactivation, and neurophysiological studies that the CA1 subregion of the hippocampus mediates the temporal processing of information within the hippocampus [2,3,4,10,15,24]. The CA1 subregion of the hippocampus has also been implicated in retrieval of contextual fear in delay as well as during trace fear conditioning paradigms [13,25,31], and has also been implicated in intermediate-term memory during a delay non-match to place task (i.e. during 5 m delays between study and test phases) [22,34]. It has also been demonstrated that CA1 lesions result in retrieval deficits in a modified Hebb Williams maze [35]. Disruption of plasticity in CA1 results in similar patterns of deficits [12,26]. Additionally, under certain conditions, disrupting plasticity of the inputs from the lateral entorhinal cortex into CA1 has been shown to disrupt the detection of visual object novelty [16]. Based on these and other findings, computational models of hippocampal function suggest that CA1 mediates the temporal processing of spatial and nonspatial information (i.e. olfactory and visual object) [11,32,33], and that CA1 is critically involved in the detection of spatial and nonspatial novelty via a match/mismatch comparison [7,8,9].

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These models overlook the subcortical outputs of CA1 (as well as the subiculum) via the dorsal fornix that have been shown to mimic the consolidation/retrieval effects seen after CA1 lesions in a spatial Hebb Williams maze [18]. These results suggest that the CA1 projections to the subcortex may participate in information consolidation/retrieval [cf. 5,36]. Among the targets of CA1 and subicular efferents via the dorsal fornix to the subcortex are the medial (and to a much lesser extent, lateral) septum, the mammillary bodies, and the anteromedial, anterodorsal, anteroventral, laterodorsal, and midline nuclei of the thalamus. It has further been shown that the medial septum sends cholinergic afferents into CA3 and CA1 via the fimbria that modulate information processing [cf. 7,8,9]. The roles of these areas for information processing have been widely studied [1,27,37].

These behavioral and computational descriptions of CA1 function, to a large degree, do not take into account the role of CA1 efferents to the septal nuclei and other subcortical brain regions along the Papez circuit (i.e. mammillary bodies and anterior thalamus) [5,29,36]. Most computational models are designed to model the architecture of the trisynaptic loop from the entorhinal cortex, through the hippocampus (dentate gyrus-CA3-CA1), and back to the entorhinal cortex. This type of analysis emphasizes the role of the Schaffer collaterals from CA3 into CA1 as well as the role of the direct perforant path from the entorhinal cortex into CA1. The readout of CA1 to the entorhinal cortex is used as an index of learning and memory.

To better evaluate the role of CA1 and subicular subcortical efferents for information processing, we developed a novel axon transection technique to selectively disrupt hippocampal efferents while leaving afferents intact (cf. figure 1A–C) [18]. This selective transection of the dorsal-most aspect of the fornix is not analogous to the fornix transections carried out previously in rodents and primates. Those manipulations were actually disruptions to all subcortical information flow in and out of the hippocampus by disrupting both hippocampal efferents and afferents in the fimbria as well as those in the fornix. A quick look at the histology figures presented in those studies suggests the fornix disruptions were usually at the level of the descending columns of the fornix, encapsulating both the fimbria and fornix (cf. figure 1D). This is not trivial since it has been shown that CA1/subicular efferents via the dorsal fornix and those from CA3 via the fimbria participate differentially in the acquisition and retrieval of maze learning [18]. The more sensitive transection protocol allows for the analysis of hippocampal subregion-specific subcortical efferents, allowing researchers to further dissect the role of hippocampal subregions for information processing.

The present experiment was designed to elucidate the role of the CA1 and subicular subcortical efferents via the dorsal fornix for temporal ordering of visual object stimuli (synonymous with judgments of recency). It was hypothesized that transecting CA1 subcortical efferents would mimic the effects of a CA1 lesion by disrupting temporal processing while leaving novelty detection intact. This hypothesis was based on experiments that showed similar behavioral deficits following dorsal fornix lesions and CA1 excitotoxic lesions [18,35]. The results, however, revealed that transecting CA1 subcortical efferents results in no change to temporal processing, but results in a clear deficit for novelty detection of visual objects. These results suggest that temporal processing is mediated by a circuit subserved by CA1 and the entorhinal cortex (i.e. via the trisynaptic loop [cf. 23]), whereas a larger circuit involving CA1 and subcortical structures may subserve novelty detection for visual objects [cf. 7,8,9], supporting computational models of both processes.

Material and Methods

Subjects

Twelve Long-Evans rats, approximately four months of age and weighing ~310 g at the start of the experiment served as subjects. The colony room was maintained on a 12 h light/dark

cycle. All rats had *ad libitum* access to water and maintained at 90–95% of their free feeding weight. All experimental protocols conformed to IACUC and AAALAC regulations. Prior to participation in the present experiment, all animals had previous behavioral experience with a modified Hebb Williams maze [18].

Surgery

Experimentally naïve rats received a selective transection of CA1 subcortical efferents in the dorsal fornix (n=6), or a control surgery (n=6). The transection protocols used for these same animals have been presented in greater detail elsewhere [18, cf. figure 1A–C]. Briefly, animals were anesthetized with a mixture of ketamine and xylazine (65 mg/kg; 10 mg/kg) and twisted bipolar stimulating/recording electrodes were lowered into the medial septum (figure 1A; AP +0.7, ML 0.1, DV ~4–5—all at a slight angle away from the midline (~5 degrees) to avoid rupturing any vasculature along bregma) and CA1 (figure 1B; AP ~3.0, ML 1–2, DV ~1.5–2). A ground screw was secured in the skull above the rostral cortex in lowered to come in contact with the dura mater. After the dura mater was carefully punctured to allow easy penetration of electrodes, potentials were alternately evoked in the medial septum from CA1 and in CA1 from the medial septum. The placements of the electrodes were adjusted to maximize this signal in both directions, and then remained untouched throughout the remainder of the surgical procedure. A retractable wire knife was lowered stereotactically until it was situated adjacent to the dorsal fornix (figure 1C; AP ~2.3, ML 0.5, DV ~2), after which the blade was protracted such that it was between CA1 and the dorsal fornix in the alveus. The knife was slowly raised until the medial septal responses evoked by CA1 stimulation were attenuated. It was then verified that stimulation of the medial septum was able to evoke responses in CA1. This was then performed on the other hemisphere, resulting in a bilateral transection of the dorsal fornix. The choice of the location of the dorsal fornix transection was such that there was never damage to CA1 or to the fimbria, also such that the transection was as rostral as possible without risking disruption of fibers from CA3 to the fimbria. Control surgery involved everything except the actual transection (i.e. the knife was lowered into place, protracted, and then immediately retracted again prior to removal).

After surgery, animals were placed in their cages on a heating pad for 1–2 hours to recover and given sweetened, crushed food, as well as given acetaminophen in their water (Children's Tylenol; 50 mL/100 mL water) for three days after surgery as an analgesic. Animals were given two weeks to recover from surgery before starting any experimentation.

Experimental Apparatus

The same rectangular chamber (35 cm wide × 92 cm long × 37 cm tall) was used for both the temporal ordering and novelty detection tasks. This size of the apparatus facilitated the animals' exploration of the objects due to the proximity to objects. The walls were made of red plexiglass and the floor was wood painted a dull grey. For this experiment, eight objects were prepared (all were three dimensional objects made of plastic, metal, or painted wood and were approximately the same size as the rat; cf. [10] for digital photographs of representative objects used in the present study). Each object was prepared in triplicate so that the animal would be unable to leave olfactory cues on an object and use that olfactory cue to guide later object exploration. Objects were grouped into sets of four objects that were used together. There were two sets of objects. Each animal received each set of objects, but which object set was used for the temporal ordering task or for the novelty detection task was pseudorandomized, as was the order the objects within a set were presented to any animal. Additionally, no animals showed excessive interest in any single object over others that would confound later measures.

Behavioral Methods

Habituation

Rats were handled by an experimenter for at least 15 min every day for five days. The day after this handling period, the animals were placed in the experimental apparatus for 30 min to habituate to the box prior to starting the experiments. The order of the two tasks given to each animal was counterbalanced.

Temporal Ordering of Visual Objects

Prior to the task, the apparatus was wiped down with an unscented cleaning solution and two copies of a given object ("A") were placed in the apparatus. Each animal was placed in the apparatus in the center and facing the long wall away from both objects and allowed to explore the objects for 5 min. After this exploration period, each animal was removed for 5 min and placed in a holding cage while the apparatus was wiped down and two copies of a second object ("B") were placed in the apparatus. Each animal was then put into the apparatus the same way as before and given 5 min to explore the "B" objects. This was repeated again for two copies of a third object ("C"). After the third set of objects was explored, the animals were placed in a holding cage for 10 min, while the apparatus was cleaned and a third copy of the first (A) and last (C) object presented were placed in the apparatus (left and right counterbalanced). Each animal was placed in the apparatus and allowed to explore for 5 min. Preferential exploration of the first object (A) over the one presented later (C) during the test session was used as a measure of intact temporal ordering.

Novelty Detection of Visual Objects

The first three object presentations in this task (i.e. A, B, C) were identical to the temporal ordering task, but with objects the animal had never seen before. After the third set of objects was explored, the animals were placed in a holding cage for 10 min, while the apparatus was cleaned and a third copy of the first (A) and a copy of a novel, never seen ("D") object were placed in the apparatus (left and right counterbalanced). Each animal was placed in the apparatus and allowed to explore for 5 min. Preferential exploration of the new object (D) over the one presented first (A) during the last session was used as the measure for intact novelty detection for visual objects.

Histology

At the conclusion of testing, each rat was deeply anesthetized with an injection of sodium pentobarbital (70 mg/kg i.p.), perfused intracardially with PBS and 10% (wt/vol) formalin, and the brain was removed from the skull and stored at 4° C in a solution of 30 % sucrose in 10% formalin (wt/vol) for 72 h prior to further processing. The brain was frozen and 40 µm sections were taken with a cryostat through the medial septum and hippocampus for Nissl (cresyl violet) and acetylcholinesterase staining.

Dependent Measures and Statistical Analysis

Time spent exploring each object was used as the dependent measure. Exploration was defined as the animal having its nose within 1 cm of the object while facing it. To determine whether there might be locomotor activity differences, each time the animal moved from one side of the apparatus to the other, a midline crossing was recorded. Additionally, exploration for each object during the presentations of each object were collected and analyzed with two-way repeated measures ANOVA (group × session) to verify that no object was explored more than any others. To control for differences in overall exploration among animals, object exploration data during the test sessions were made into a ratio score to constrain the values between -1 and 1. For temporal ordering, the ratio was exploration of the first object minus the exploration

of the latter object divided by the sum of the two $[(A-C)/(A+C)]$. For the novelty detection task, the ratio was the exploration of the new object minus the exploration of the first object divided by the sum of the two $[(D-A)/(D+A)]$. For both ratios, no preference for one object over the other resulted in a ratio value near zero. Preference for one object over another is reflected in a value approaching 1 or -1 [9,13,14]. As the ratios are defined, for temporal ordering, intact temporal ordering (favoring primacy over recency) results in a positive value. In the same vein, intact novelty detection (favoring novelty over familiarity) results in a positive value. Prior to any analyses on the ratios, it was verified that the control group showed a preference for one object over the other by means of a one-tailed t-test (i.e. the null hypothesis was that the control groups would show a nonzero, positive value). A one-way ANOVA with groups (dorsal fornix transection, control) as the between factor was performed on the temporal ordering and the novelty detection ratios. All effects were considered significant at $p < 0.05$ (note: for $n=6$ per group, statistical power was maintained >0.80 for $p < 0.05$).

Results

Histology

The results of the dorsal fornix transections are depicted in figure 2. More thorough anatomical, neurophysiological, and neurochemical analyses of the lesions of these same animals have been reported elsewhere [18]. In short, the responses in the medial septum from CA1 stimulation were reduced by the transection, whereas the CA1 responses from medial septal stimulation were unchanged. Anatomical tract tracing experiments reported in [18] revealed that the dorsal fornix transection disrupted ~50% of CA1 subcortical efferents, without significantly disrupting projections from the medial septum into the hippocampus. It should be noted that since only 50% of these efferents were transected, the dorsal fornix transection was not complete, it just greatly reduced the CA1 subcortical outputs via the dorsal fornix. There was no disruption in acetylcholinesterase staining in the hippocampus after the transection, additionally suggesting that there was no large-scale disruption to the projections from the medial septum into the hippocampus.

Behavior

To determine if there were differences in baseline locomotor behavior, midline crossings were recorded. There were no significant differences between the two groups on any of the trials presented during both tasks for locomotor activity (all $p > 0.2$; table 1). These data suggest that all of the animals entered each side of the apparatus similarly, so any differences in object exploration were due to actual differences in object exploration, and not confounded by locomotor differences. Also, no side preferences were observed that would confound exploration measures (data not shown). Furthermore, no significant differences were found between the animals' exploration of object A, B, or C during the object presentation sessions (all $p > 0.2$; table 1)

The data for the temporal order for visual objects task is presented in figure 3. Both control and dorsal fornix transection groups showed significant preference for the first object (for control, $t(5)=2.90$, $p < 0.05$; for dorsal fornix transection $t(5)=6.71$, $p < 0.05$). These data were grouped, and one-way ANOVA were performed. There was no significant difference between the dorsal fornix transection group and the control group for temporal processing of visual objects, in fact, the dorsal fornix transection group showed a nonsignificant trend toward showing a greater preference for the earlier object over the latter relative to the control group ($F(1,10)=4.13$, $p=0.07$; mean ratios 0.39 ± 0.09 for control and 0.59 ± 0.12 for dorsal fornix transection group).

The data for the novelty detection for visual objects task is presented in figure 3. The control transection group showed significant preference for the novel object (control, $t(5)=5.384$, $p<0.05$, whereas the dorsal fornix transection group did not ($t(5)=-1.270$, $p>0.05$). These data were grouped, and one-way ANOVA were performed. There was a significant difference between the dorsal fornix transection group and the control group for novelty detection of visual objects ($F(1,10)=17.05$, $p=0.002$). These data suggest that the animals with dorsal fornix transections were unable to either properly process or respond to visual object novelty (mean ratio 0.52 ± 0.1 for control and -0.09 ± 0.11 for the dorsal fornix transection group).

Discussion

CA1 lesions result in deficits for temporal ordering of visual objects, odors, and spatial locations [15]. Inactivation of the inputs to CA1 from the entorhinal cortex causes deficits for novelty detection of visual objects, but only when plasticity in the lateral perforant path inputs is disrupted by infusions of naloxone, suggesting that CA1 itself may be involved in visual object novelty detection [16]. Furthermore, disrupting the septal cholinergic inputs of the hippocampus by infusions of scopolamine into CA3 has been shown to cause deficits for visual object novelty detection [17].

The data from the present experiment suggest that disrupting CA1 subcortical outputs result in deficits for visual object novelty detection without concomitant disruptions for the temporal processing of visual objects. Importantly, since the animals with dorsal fornix transections were able to process temporal order or judge recency, it is reasonable to assume that a simple deficit in object recognition could not underlie the novel object exploration deficits seen in the present study. These data also suggest that temporal ordering (judging recency) may be subserved by a separate mechanism than that underlying detection of novelty. In other words, judging between the relative strengths of memory traces is not the same thing as judging between the presence/absence of a memory trace.

These results are congruent with the idea of separate match/mismatch processes underlying novelty detection [7,8,9] and temporal processing mechanisms underlying temporal ordering or recency judgments [11,24,32,33]. In fact separate, independent mechanisms underlying these two processes have been proposed (cf. [7,8,9,11,24]). Actually, these two processes appear not to be mutually exclusive since distinct neural coding schemes appear to underlie each process (i.e. two different neural codes that may co-occur). Match/mismatch processing appears to involve hippocampal-subcortical loops to modulate the activity of the hippocampus to facilitate encoding or retrieval based on the CA1 activity state [8,9], whereas temporal processing appears to involve gradual changes to the overall CA1 activity patterns that can be decoded by the entorhinal or other cortices [11,24]. Alternatively, novelty detection for visual objects may involve additional subcortical structures along the Papez circuit, including the mammillary bodies and anterior thalamic regions (cf. figure 4), or else cholinergic feedback from the medial septum.

The present data can be fit into this theoretical framework by considering the differential effects of CA1 lesions [10,16] and disruption of CA1 subcortical efferents (present study). CA1 lesions result in alterations to temporal processing (i.e. the switch from a preference for primacy to preference for recency), but intact novelty detection. In light of the mechanism underlying temporal processing [11,24] in the absence of CA1, there is no output to the entorhinal cortex that can be used to make a judgment of temporal order, so other regions that also may underlie temporal processing in parallel with CA1 mediate these processes based primarily on recency judgments (e.g. perirhinal and prefrontal cortex [6,14,27]). However, for novelty detection, the above mechanism is not used to determine whether an object is novel. In this sense, if the CA1 and subicular efferents to the subcortex participate more in novelty detection than in temporal

ordering, then a dorsal fornix transection would greatly reduce the information from CA1 (by ~50% based on tracing studies), thus making novelty detection inefficient.

Based upon these theoretical models and the data, we propose that when the subcortical projections from CA1 are disrupted by a dorsal fornix transection and the Schaffer collateral and direct perforant path inputs to CA1 are maintained; any gradual changes in CA1 activity patterns are projected to the entorhinal cortex to be used for temporal processing. For novelty detection, CA1 is intact, but unable to effectively communicate information based on match/mismatch processes to the septum and/or subcortical structures along the Papez circuit [29]. This is different than a lesion of CA1 because CA1 processing is intact, and can “normally” influence all the cortical targets, but cannot “normally” influence subcortical targets. The primary assumption of this analysis is that lesion to CA1 results in a disconnection of a portion of a circuit, so the brain may compensate by bypassing CA1 when possible (i.e. via recruitment of the subiculum if appropriate or else recruitment of the perirhinal cortex). When a subset of CA1 efferents is disrupted, either by chemical inactivation or transection, the actual circuit is sufficiently intact that the brain does not bypass that portion of the circuit, but the circuit is nonetheless altered. In this way, a different network may be disrupted by the dorsal fornix transection than by a lesion. In other words, a CA1 lesion may disrupt the trisynaptic loop, whereas a transection of CA1 subcortical efferents would disrupt a larger network of structures along the Papez circuit [29].

The data can also be interpreted two additional ways: that a disruption of septal inputs from the hippocampus results in abnormal anxiety/neophobic behaviors that fundamentally altered the way the animals explored or reacted to novelty, or that the animals completely forgot object A by the time the test was presented. Although no data were collected to explicitly refute or control for the first alternative, a separate study involving these same animals investigating classical fear conditioning suggests that these animals suffer a decreased level of fear related behaviors, as opposed to an increase in fear-related behaviors [19]. The second alternative suggests that the animals with dorsal fornix transections have forgotten object A by the time the preference test was presented, resulting in a novelty-detection like preference for A over C in the temporal ordering task and no preference for A or D (as they would both be novel), is intriguing. This alternative has the benefit of explaining the pattern of data in the present experiment, but previous work with these same animals [18] suggests that the animals are fully capable of learning within a day (and almost learning faster than control animals), but have problems with retention 24 hr later.

We suggest that the deficits we see are not due to any sort of enhanced forgetting, but due to an alteration in information processing. We base this both on previous data as well as on the fact that these animals do not show the same level of preference for A over C in the temporal task that control animals show for novelty (~0.6 vs. ~0.3). It is fully possible, however, that the animals do show a subtle alteration in behavior that results in enhanced preferences for one object over the other, and that that may contribute to the pattern of results presented in this study.

Additionally, the lack of a CA1 lesion-induced effect for novelty detection may also be attributable to the limitations in the lesion methods used. Closer inspection of the lesions reported by [14,15] and earlier studies (cf. [4,10,13,20,21,22,23,31,34]) suggest that the distal-most portion of CA1 (furthest from CA2; the area adjacent to the subiculum) and the subiculum are spared, the portion of CA1 that has been shown to receive the greatest level of lateral entorhinal and perirhinal cortex inputs. If this portion of CA1 would have a greater role in the processing of novelty than temporal processing, then these effects would not be present in the CA1 lesions as reported previously by our lab. Additionally, it is also highly probable that the

dorsal fornix transections further affected efferents from the subiculum to the subcortex, further exacerbating any effects of the transection.

In summary, these data suggest that 1) disrupting the subcortical outputs of CA1 act differentially than excitotoxic lesions to CA1, and that 2) novelty detection and temporal processing for visual objects are not mutually exclusive processes, and may be processed by CA1 in parallel. These data provide support for both models of match/mismatch models of novelty detection and temporal processing as previously described [7,8,11,24]. Future work is needed to evaluate the role of the CA1 outputs to the entorhinal cortex for temporal ordering to fully validate the present analysis of CA1 function. The present pattern of results in conjunction with the effects of full CA1 lesions needs to be integrated into future models of CA1 function as well as interpretation of previous effects of CA1 disruption.

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Figure 1. Dorsal Fornix Anatomy

A. Medial septum recording/stimulating site with typical stimulating/recording electrode placement marked. **B.** CA1 recording/stimulating site with typical stimulating/recording electrode placement marked. **C.** Site of dorsal fornix transection in [17] with the knife placement and orientation depicted. **D.** The dorsal fornix is shaded black with grey fill and the fimbria is shaded grey with black stripes. Traditional fornix lesions generally ablate both the fornix as well as the fimbria. The present manipulation only disrupts the dorsal fornix, but leaves the fimbria intact.

Figure 2. Histology

A. Photomicrograph of a dorsal fornix transection in an acetylcholinesterase-stained section.

B. Diagram of the largest (grey) and smallest (black) dorsal fornix transection on a plate modified from Paxinos and Watson [28]. More detailed analyses of these lesions are available in Hunsaker et al. [18].

Figure 3. Temporal Ordering and Novelty Detection for Visual Objects

There is no significant difference between control animals and animals with dorsal fornix transections for temporal ordering for visual objects, although there is a modest enhancement of temporal ordering after dorsal fornix transections that approaches significance (i.e. a ratio score closer to 1; $p=0.07$). Animals with dorsal fornix transections show a significant deficit for novelty detection for visual objects relative to control animals (i.e. a ratio score closer to 0; $p=0.002$), they also show no preference for novelty since they have a ratio score near zero ($p>0.05$).

Figure 4. Diagram of hippocampal projections to the subcortex via the dorsal fornix

Black arrows denote projections altered after dorsal fornix transections. The grey arrows denote the trisynaptic loop, and the white arrows denote other projections.

Table 1
Object Exploration and Midline Crossings Raw Values

Object Exploration (seconds)								
	Temporal Order				Novelty Detection			
	A	B	C	A C	A	B	C	A D
Control	24 +/- 3	26 +/- 4	22 +/- 4	11 3	23 +/- 5	24 +/- 1	24 +/- 5	15 11
Dorsal Fornix	27 +/- 5	24 +/- 3	25 +/- 2	13 7	24 +/- 5	26 +/- 7	25 +/- 2	09 15
Midline Crossings								
	Temporal Order				Novelty Detection			
	A	B	C	A C	A	B	C	A D
Control	15 +/- 4	17 +/- 7	14 +/- 3	11 +/- 4	16 +/- 7	11 +/- 4	13 +/- 3	12 +/- 5
Dorsal Fornix	18 +/- 3	15 +/- 1	15 +/- 5	14 +/- 5	15 +/- 1	14 +/- 5	16 +/- 2	14 +/- 2

Object exploration values and midline crossings for each object presentation session (A, B, C, Test) during temporal order and novel object detection tasks (presented as means +/- standard errors). In no cases were there differences between groups or between sessions (all ps>0.2). Of note is the relative homogeneity of exploration and midline crossing scores that may be influenced by the small size of the box.