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Deficits in Landmark Navigation and Path Integration after Lesions of the Interpeduncular Nucleus

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Abstract

Experiments were designed to determine the role of the interpeduncular nucleus (IPN) in three forms of navigation: beacon, landmark, and path integration. In beacon navigation, animals reach goals using cues directly associated with them, whereas in landmark navigation animals use external cues to determine a direction and distance to goals. Path integration refers to the use of self-movement cues to obtain a trajectory to a goal. IPN-lesioned rats were tested in a food-carrying task where they searched for food in an open-field, and returned to a refuge after finding the food. Landmark navigation was evaluated during trials performed under lighted conditions and path integration was tested under darkened conditions, thus eliminating external cues. We report that IPN lesions increased the number of errors and reduced heading accuracy under both lighted and darkened conditions. Tests using a Morris water maze procedure indicated that IPN lesions produced moderate impairments in the landmark version of the water task, but left beacon navigation intact. These findings suggest that the IPN plays a fundamental role in landmark navigation and path integration.

Keywords

dorsal tegmental nucleus; habenula; head direction cells; navigation; rat; spatial orientation

Introduction

Animals can use a variety of distinct navigational strategies and stimulus types to locate goals. For example, they can follow odor paths deposited by themselves or by their conspecifics, or simply move towards visual cues directly above or besides a goal location (Redhead et al., 1997; Wallace et al., 2002). This form of navigation has been referred to as beacon (or taxon) navigation (Gallistel, 1990; O'Keefe & Nadel, 1978). Alternatively, animals can learn the direction and distance of a goal in relation to a visual cue or a configuration of several visual cues, even if the goal is hidden (Morris, 1981; Prados & Trobalon, 1998; Roberts & Pearce, 1998). Strategies based on such cues are often referred to as landmark (or locale or piloting) navigation (Gallistel, 1990; O'Keefe & Nadel, 1978). Although beacon or landmark cues are generally reliable, they can become unreliable if they

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change over a day or season and are also uninformative if the animal is in a novel environment. Under these circumstances, animals can keep track of their own self-movement cues (i.e., vestibular, optic flow, motor efference copy, and proprioceptive cues), which they can integrate in relation to a known starting point to determine a current location or plot a trajectory to a goal. This form of navigation is often called path integration (or dead reckoning), and has been demonstrated in a wide range of animals including humans (Etienne & Jeffery, 2004; Gallistel, 1990).

Research on the neurobiological basis of navigation has centered on the role of cortical and subcortical limbic brain regions, mostly due to the findings of spatially responsive neurons in these areas. For instance, the CA1 and CA3 subfields of the hippocampus contain cells that are active when an animal is located in a specific place in an environment (Best et al., 2001; O'Keefe & Dostrovsky, 1971). These neurons are termed place cells and have been observed in rats, mice, and primates (Ekstrom et al. 2003; Kentros et al., 2004; Ludvig et al., 2004; Ono et al., 1993). In recent years, Moser and colleagues have identified populations of spatially responsive cells in the medial entorhinal cortex which comprises the major cortical afferents of the rat hippocampus (Hafting et al., 2005; Moser et al., 2008; Sargolini et al., 2006). These neurons, which are known as grid cells, are active in multiple locations of an environment such that their firing fields form a grid-like pattern. A third class of spatial cells can be found in the postsubiculum of the hippocampal formation (Taube et al., 1990a; 1990b), as well as in several interconnected subcortical limbic structures, including the anterior thalamus, lateral mammillary nuclei, and dorsal tegmental nuclei (see Taube, 2007 for a review). These cells discharge as a function of an animal's head direction (HD), but their activity is independent of the animal's location and on-going behavior. HD cells have been observed in several species including rats, mice, chinchillas, guinea pigs, and non-human primates (Taube, 2007). Collectively, place, grid, and HD cells provide animals with an abundant source of spatial information for determining their current location and direction. It is well known that lesions of brain regions containing these cell types produce navigational impairments in a wide range of path integration and landmark-based spatial tasks (Aggleton et al., 1996; Frohardt et al., 2006; Maaswinkel et al., 1999; Morris et al., 1982; Parron & Save, 2004; Steffenach et al., 2005; Taube et al., 1992; Whishaw et al., 2001). Thus, these findings suggest that the spatially modulated place, grid, and HD cell systems likely play a fundamental role in navigation.

In recent years, research has focused on the role of subcortical brain regions in spatial navigation and in the generation and maintenance of hippocampal and limbic system place and HD cells (Bassett et al., 2007; Blair et al., 1999; Calton et al., 2003; Clark et al., 2008; Frohardt et al., 2006; Sharp & Koester, 2008). The interpeduncular nucleus (IPN), a midline structure located on the ventral surface of the midbrain, is of particular interest because it occupies a pivotal position between limbic midbrain and brainstem structures (Klemm, 2004; Morley, 1986; Sutherland, 1982). Interestingly, the IPN is reciprocally connected to HD cell circuitry, specifically the dorsal tegmental nuclei, and sends sparse inputs to the dentate and CA3 subfields of the hippocampus (Baisden et al., 1979; Groenewegen et al., 1986; Montone et al., 1988; Segal, 1975; Shibata & Suzuki, 1984). Although there has been no attention directed toward the role of the IPN in navigation, large lesions of its primary afferent, the habenula, produce impairments in landmark navigation tasks (Lecourtier et al., 2004). Furthermore, Sharp et al. (2006) reported that some cells in the IPN and habenula contained movement-related correlates associated with angular head velocity or linear running speed. Moreover, we recently found that selective lesions of the IPN significantly reduce the extent of direction-specific firing of HD cells as well as lessen the amount of control exerted by landmarks and self-movement cues (Clark et al., 2009). Such findings indicate that the IPN may convey spatially important information to HD cell circuitry and may play a fundamental role in navigation.

Given the studies described above, we were interested in examining whether the IPN is involved in beacon, landmark, or path integration based navigation. To address this issue we lesioned the IPN in rats and tested them in two commonly used spatial tasks: the food-carrying and Morris water tasks (Frohardt et al., 2006; Maaswinkel et al., 1999; Morris, 1984; Parron & Save, 2004; Whishaw & Tomie 1997; Whishaw et al, 2001). First, we tested IPN lesioned and intact rats in the food-carrying paradigm in which they searched for large food pellets in an open-field, and directly returned to their home refuge after finding the food. Using this task, we evaluated the animal's ability to return to the home refuge when the testing room lights were turned off, thereby removing visual landmark and optic flow information. Thus, because external cues were not useful in this version of the task, rats had to rely on their ability to integrate self-movement information from their starting location to return to the home refuge. Following this path integration test, the room lights were turned back on and the rats were then allowed to use the visual room cues to navigate to the home refuge. Finally, we tested the same rats in a Morris water maze procedure in which they were first trained to escape from cool water by navigating toward a visual cue (a beacon) marking a hidden platform. Rats were then tested in a standard hidden platform procedure in which they were required to learn the relationship between the visual room cues and the platform location. This latter test provided a second evaluation of landmark navigation. Below we report that IPN lesioned rats were impaired in both landmark navigation and path integration, but were unimpaired in navigating to the goal when it was marked with a beacon.

Methods

Subjects

Subjects were 19 female Long-Evans rats, weighing 250-300 g at the beginning of testing. Rats were singly housed in Plexiglas cages and maintained on a 12 hr light/dark cycle. All procedures involving the rats were performed in compliance with institutional standards as set forth by the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and the Society of Neuroscience.

Surgery

All animals were anaesthetized with Nembutal (40 mg/kg, i.p.) and given atropine sulfate (5 mg/kg, i.p.) to prevent respiratory distress. The animals were placed in a Kopf stereotaxic instrument (David Kopf Instruments, Tujunga, CA) and an incision was made to expose the skull. Small holes were then drilled into the skull above the IPN. Neurotoxic lesions of the IPN ($n = 10$) were produced by infusing 0.15 μ l of a 100mM solution of N-methyl D-aspartate (NMDA; dissolved in 0.9% saline) into six mid-line sites of the brain. The solution was infused at a rate of 0.02 μ l/min through a 1 μ l Hamilton syringe (Hamilton Company, Reno, NV). Before infusing the NMDA solution, the syringe rested at each injection site for 3 min; this allowed the tissue to settle around the syringe needle. After each injection, the syringe was left in place for 5 min before being slowly removed. The needle was wiped with distilled water in between each injection; this reduced overlying cortical damage. Neurotoxic lesions were produced at six rostral-caudal locations based on coordinates provided by Paxinos and Watson (1998). Each site was 2.1 mm lateral to bregma and at a 14° angle from vertical in the coronal plane: posterior (P) = -5.6 and ventral (v) = -8.8 mm; p = -6.0 and v = -8.8 mm; p = -6.4 and v = -8.8 mm; p = -6.8 and v = -9.36 mm; p = -7.2 and v = -9.36 mm; p = -7.4 and v = -9.36 mm. Sham rats ($n = 9$) were given identical procedures but did not receive infusions of NMDA into the IPN.

Feeding

Water was provided *ad libitum* while access to food was restricted as necessary to maintain the animal's body weight at 80% of its free feeding weight. They were placed on an *ad lib* food diet for a 1-week period immediately after surgery and during training in the water task.

Apparatus

Foraging apparatus—The food-carrying apparatus was identical to that used by Frohardt et al (2006). Briefly, the apparatus consisted of a large, gray, circular (180 cm diameter) open field with 16 symmetrically placed black food cups on the surface (Fig. 1). Each food cup could be baited with a large 750 mg sugar pellet (Bioserv, Frenchtown, NJ). Rats have a reliable proclivity to carry these pellets back to their refuge for eating, rather than consuming them at the food cup (Whishaw et al., 1990). Intact rats normally carry the food pellets directly back to the refuge in a relatively straight line. The entire field was supported by four rolling casters and was mounted on a central bearing that allowed the field to be rotated around a central platform. The central platform was slightly raised (1 cm thick, 53.3 cm in diameter) and remained stationary throughout the experiment. The field was surrounded by a wall (38 cm high) containing eight uniformly distributed doorways (each 13 cm wide) that were covered by a black curtain that could be parted in the middle to allow the rat to go through. There was a refuge behind one doorway (13 cm wide, 40 cm long, 30.5 cm high) that was completely covered with a cardboard lid, and served as a place of safety and familiarity for the rat. The other seven doorways served as false refuges and had wooden barriers behind the curtains. The foraging apparatus was surrounded by black floor-to-ceiling curtains (250 cm in diameter). A large white floor-to-ceiling curtain that covered ~80° of arc served as a visual landmark and was placed ~135° opposite the refuge. Four uniformly arranged lamps were located above the apparatus to provide illumination. An infrared video camera was located 3 m above the center of the field so that the behavior of the rats could be videotaped when the room was either illuminated or when the room lights were turned off.

Swimming pool—The swimming pool was a 180 cm diameter and 50 cm high round white tub positioned 14cm above the floor. The pool was filled to a depth of 23 cm with 21–22° C water. The water was made opaque by the addition of 750 ml of powdered white paint. A clear Plexiglas platform with a 13 cm diameter top was placed in the pool such that the top of the platform was 1 cm below the surface of the water. A video camera was located above the center of the swimming pool so that the behavior of the rats could be videotaped and analyzed. The swimming pool was located in a test room in which many cues, including counters, cupboards, and posters were located; however, it was possible to eliminate these cues by hanging black floor-to-ceiling curtains around the pool (250 cm in diameter).

Procedure

Food carrying task—All rats received training in the food-carrying apparatus (Fig. 1) before surgery was performed. At the start of training, all of the food cups were baited with pellets, and each rat was placed in the refuge and allowed to forage for the pellets. A single food carrying trial consisted of the rat leaving the refuge, finding the food pellet, and carrying it back to the refuge to eat it. Similar to previous observations, once a rat found a pellet it typically returned to the refuge to eat it (Frohardt et al., 2006; Whishaw & Tomie, 1997). However, when rats tried to eat a pellet in the open field, or search for another pellet during a return path, the behavior was discouraged by making a startling noise (shaking keys) or taking the food pellet away. Animals were allowed to retrieve and eat four pellets per day. As the rats became more proficient at the task, the number of baited food cups was

reduced daily until there was only one pellet available per trial in one of four centrally located food dishes. Pre-training was complete once the rats completed four successive retrieval trials and reliably returned directly to the correct refuge for two consecutive days. Once training was finished, rats were divided into two groups (IPN lesion and Sham) matched to minimize behavioral differences. After recovery from surgery (~1 week), rats received food carrying trials first with the room lights turned off, and then with the room lights turned back on.

Lights-Off—This portion of the task assessed whether the IPN-lesioned rats could accurately return to the refuge using path integration based processes. Thus, various attempts were made to prevent the use of external allothetic information such as visual, olfactory, and auditory cues. First, similar to several previous studies (Wallace et al., 2002; Whishaw et al., 2001), we prevented the use of visual cues by turning the room lights off and sealing the room to block all visible light. In the dark, an infrared camera was used to record behavior. Infrared is a light wave in which rats are unable to see (Neitz & Jacobs, 1986). Two infrared emitters were attached on different walls providing sufficient infrared illumination in the room so the rat was visible on the camera. To further ensure that the rats did not use visual cues during lights-off sessions, the refuge was moved to a different location at the beginning of each trial (Fig. 1B). To eliminate the use of olfactory cues as a source of guidance, the floor of the open field was cleaned with 80% ethanol and rotated after each trial. Previous reports have shown this procedure to be an effective method for preventing the use of surface cues (Frohardt et al., 2006; Maaswinkel et al., 1999). In addition, a radio tuned to an FM station that played white noise was placed next to the overhead camera. This provided background noise to eliminate potential auditory cues. Under these conditions, rats performed 12 food carrying trials across 3 consecutive days (4 trials/day). For each trial, the food pellet was placed pseudorandomly in one of four centrally located food dishes. The placement was consistent between rats, but not between trials. Daily food carrying sessions were video recorded with the infrared camera and the behavioral analysis was performed offline.

Lights-On test—This portion of the task assessed whether the IPN-lesioned rats could use landmarks to accurately return to the refuge. Thus, in contrast to lights-off testing, the room lights were left on, and the refuge remained in the same location throughout testing (Fig 1B). Rats performed 12 trials across 3 consecutive days under these conditions (4 trials/day). Similar to the trials under the infrared condition, food pellets were placed pseudorandomly in one of four centrally located food dishes. Again, the placement was consistent between rats, but not between trials. To ensure that task difficulty did not vary, the pattern of food placement in this version of the task was identical to that of the infrared trials. Olfactory and auditory cues were again obscured by cleaning the floor of the open field with 80% ethanol and a dry cloth after each trial and by using a radio tuned to an FM station placed near the camera. Daily food carrying sessions were recorded with the overhead camera and the behavioral analysis was performed offline.

Cued platform swimming task—Following testing in the food-carrying tasks, rats were trained in a cued platform version of the water task. This test examined the ability of the rats to swim toward a cue directly associated with the platform location, i.e., beacon navigation. The visual cue used in the present study was a flag mounted on a wooden dowel that stood 13 cm above the platform (Taube et al., 1992). The flag consisted of four pieces of black cardboard attached at 90° angles making it clearly visible from any point in the pool. To ensure that only the flag was used to locate the platform, various attempts were made to obscure the room cues (i.e., posters, counters, cupboards, etc.) as well as the apparatus cues (i.e., pool walls), which all can act as spatial cues (Hamilton et al., 2007; 2008; Hoh & Cain,

1997; Morris, 1984). First, to prevent the use of room cues, training took place with the black floor-to-ceiling curtains surrounding the swimming pool. To prevent the use of the apparatus walls as a spatial cue, the platform was moved between 12 different pool locations after each swimming trial (e.g., the center of the pool quadrants, center of the pool, and locations near and away from the edge of the pool). Animals were trained for two days, each day consisting of two blocks of four consecutive trials (4 hr interval between daily training blocks and 24 hr between days). A trial consisted of placing a rat by hand into the water facing the wall of the pool at one of four pseudorandomly selected starting positions (north, south, east, and west) around the perimeter of the pool. Rats were allowed to swim until they found the platform or until 60 sec elapsed. If a rat found the platform in less than 60 sec, it was permitted to remain on the platform for 30 sec. If after 60 sec the rat failed to find the platform, it was guided to the platform and permitted to remain there for 30 sec. At the end of the trial the rat was returned to a holding cage, and approximately 10–20 min elapsed, during which the remaining rats in the cohort were tested before beginning the next trial. After four trials, the animals were returned to their home cages and the same procedure was repeated for the next block of trials.

Hidden platform swimming task—After training in the cued variant of the water maze task, rats were trained in a hidden platform version. The purpose of this variant of the task was to assess the rat's ability to learn the fixed spatial relationship between the room/apparatus cues and the hidden platform. This task provided a second assessment of landmark navigation. Thus, the floor-to-ceiling curtains were removed to allow the animals to view the distal room cues. Moreover, the flag was removed from the platform and the platform remained in the same location throughout training. Animals were trained for four days, each day consisting of two blocks of four consecutive trials (4 hr interval between daily training blocks and 24 hr between days). Trials were similar to the non-spatial cued training, consisting of placing a rat by hand into the water facing the wall of the pool at one of the four starting positions (trial length 60 sec; time on platform 30 sec). Again, at the end of each block of trials, rats were returned to their home cages and the same procedure was repeated during the next training block. A probe trial with the platform removed from the swimming pool was conducted for 60 sec at the beginning of the fifth day. For the probe trial, the rats were released from the side of the pool opposite of the platform.

Behavioral analysis

Food carrying task—Individual food carrying trials were segmented into searches and returns (Frohardt et al., 2006; Wallace et al., 2002; Whishaw & Tomie 1997). Searches were defined as the moment the rat left the refuge to the point at which the rat finds and picks up the food pellet. The return segment was defined as the point in which the rat picks up the food pellet and carries it back to the refuge. Using a stopwatch, the duration of searches and returns were measured in seconds. Because some recent evidence suggests that rats can use other sources of information to correct and/or change their trajectory after initially orienting to a goal (Hamilton et al., 2004), returns were further analyzed by measuring the initial and final heading angles. The initial heading angle was defined as the angle between the refuge and the rat's directional heading when the rat picked up the food pellet and was one body length away from the food cup. The final heading angle was defined as the angle between the refuge and the rat's directional heading when the rat approached the refuge and crossed a "virtual finish line" that was 2 cm from the outside wall of the apparatus. Each angle was measured with a resolution of 6° that was either clockwise or counterclockwise from the refuge. For analysis, the initial and final heading angles were converted to absolute values; thus, the maximum deviation was 180°. Finally, returns were measured for accuracy by indicating the first doorway the rat entered. A measure of choice accuracy was calculated by counting the number of times the rat chose the correct refuge location. Mean search

duration, return duration, initial heading, final heading, and the number of correct choices were calculated for each testing day and animal.

Swimming task—Each training trial for the cued and hidden platform tasks was measured for duration (seconds) using a stopwatch. Mean escape latencies for each animal were calculated for each block of four trials. For the probe trial, the pool was divided into four equal quadrants (see inset of Fig. 8A) and the percentage of time that rats spent in each quadrant was measured. Because previous work has shown that rats can use the distance from the pool wall to localize the platform position (Hamilton et al., 2007;2008;Hoh & Cain, 1997), we calculated the percentage of time that rats spent in an annulus marking the platform positions relative to the pool wall and two other equal annuli marking the middle and outer segments of the swimming pool (see inset of Fig. 8B). Thus, if the animals generally learned that the platform was a particular distance from the pool wall, then we would see a greater percentage of swim time in the platform annulus.

Statistics

Analysis in the food-carrying and water maze tasks was performed using a two-factor repeated measures analysis of variance (ANOVA) with Group as between-subject factors and Days/Block as within-subjects factors. Follow-up simple contrasts as well as mean comparisons were also performed (Tabachnik & Fidell, 2007).

Histological analysis

At the completion of the experiments, animals were deeply anaesthetized with sodium pentobarbital. The rats were then perfused intracardially with saline followed by a 10% formalin solution. Each brain was removed from the skull and was post-fixed in a 10% formalin solution for at least 24 hr. The brains were then cryoprotected in a 20% sucrose solution for 24 hr, and were then frozen and cut coronally at 30 μ m sections with a cryostat. Every third section was taken through the IPN and mounted on glass microscope slides. Sections were stained with thionin, and examined under light microscopy to evaluate the lesions. To quantify the extent of electrolytic and neurotoxic damage to the IPN, digital images were captured at three rostral-caudal levels (-5.8 mm, -6.3 mm, and -6.8 mm posterior to bregma) provided by Paxinos and Watson (1998). The area of undamaged tissue in the IPN was calculated at each rostral-caudal level using Image-J software (<http://rsb.info.nih.gov/ij/index.html>). Tissue was considered undamaged if it contained healthy neurons and few glial cells. Once the area of undamaged tissue was calculated, the area of spared tissue was summed across the three sections and compared with an average area measured in the control rats. The total amount of damage was calculated using the following formula: Tissue damaged = [average area of IPN in control rats (pixels²) - total area of spared IPN tissue in lesioned rats (pixels²) / average area of IPN in control rats (pixels²)] $\times$ 100%

Results

Histology

Figure 2A shows the rostral-caudal extent of the largest and smallest IPN lesions at select plates from Paxinos and Watson (1998). Representative sections from Sham and IPN-lesioned rats are shown in Figure 2B and 2C, respectively. Overall, the amount of damage to the IPN varied from 78 to 100%. Seven rats sustained a 90-100% complete lesion of the IPN, while three rats had 78, 84, and 88% damage, respectively. The variability in the lesions was mainly due to the fact that the rats had some sparing in the most caudal regions of the IPN. Inspection of the histology indicated that all of the lesioned rats showed complete damage to the rostral region of the IPN, and all but three animals had complete

damage to the caudal portions of the IPN. It was particularly important that rostral areas were completely lesioned since the rostral subnuclei of the IPN are prominently connected with the dorsal tegmental nuclei and thus capable of influencing HD cell activity (Groenewegen et al., 1986; Hemminger & Moore, 1984; Liu et al., 1984). Because there were no observable behavioral differences (i.e., measures of return accuracy) between the animals with smaller lesions and those with larger lesions, we pooled all of the lesioned rats into one group.

The lesions typically extended into lateral and dorsal structures, including the paranigral nucleus, interfascicular nucleus, caudal linear nucleus of the raphe, and the ventral tegmental area (see Fig. 2C). However, in all cases, damage to these adjacent structures was incomplete and unilateral (<20%). Three IPN lesioned and four Sham animals had minor unilateral damage (<30%) to the overlying cortex bordering the dysgranular region of the retrosplenial and the medial subregions of the parietal cortex. Previous studies have reported that neurotoxic damage to the retrosplenial or parietal cortices can produce impairments in spatial tasks (Aggleton & Vann, 2004; Harker & Whishaw, 2004; Parron & Save, 2004). However, because very large lesions of these cortical structures are required to produce impairments, it is unlikely that our <30% damage contributed to the results in the present study. Nevertheless, to rule out this possibility, we compared the pre and post-surgical behavioral data from the food carrying task for the four cortically damaged Sham animals. This analysis did not reveal any significant differences on any of our behavioral measures ($p > .05$). Thus, we argue that lesions specific to the IPN are the most likely cause of our experimental findings.

Lights-Off test

The purpose of the lights-off task was to evaluate the navigational accuracy of IPN-lesioned rats when they are limited to using a path integration based strategy. We first examined the search patterns of rats during infrared conditions. Because one of the Sham rats failed to carry food on the final lights-off testing day, data from this animal's final testing session was excluded from statistical analysis. The left panels in Figure 3 show the search paths of representative rats from Sham and IPN-lesioned groups for the first infrared test trial. Notice that the search paths of rats from both groups have similar circuitous patterns that reach large portions of the apparatus. The time taken to locate a food pellet was used to measure performance during searches and is shown in Table 1. On average, the search times of IPN-lesioned rats were similar to that of Shams. A repeated measures ANOVA on the search times did not indicate any group ($F(1, 16) = .142, p = .711$), day ($F(2, 32) = 1.02, p = .371$), or group by day interactions ($F(2, 32) = 1.82, p = .179$).

Although no group differences were observed during search behavior in the infrared test, the accuracy of returns varied across the groups. As shown in the right panels of Figure 3, the return paths of IPN-lesioned rats were less accurate, less direct, and longer than the return segments of Sham rats. Indeed, IPN-lesioned rats chose the correct refuge $1.83 \pm .24$ times out of four possible attempts, while Sham animals chose the correct refuge nearly twice as often $3.33 \pm .13$ times (Fig. 4A). An ANOVA confirmed that the lesioned rats chose the correct refuge significantly less than Shams ($F(1, 16) = 17.5, p = .001$). The ANOVA did not indicate a day ($F(2, 32) = .711, p = .499$), or group by day interaction ($F(2, 32) = 1.12, p = .338$). A similar result was obtained when evaluating the time taken to return to the refuge with the food pellet (Fig. 4B). A repeated measures ANOVA conducted on return time revealed that the lesioned animals had greater return durations, as indicated by a significant group effect ($F(1, 16) = 21.2, p < .001$). An ANOVA did not indicate any significant day ($F(2, 32) = 1.32, p = .282$), or group by day interactions ($F(2, 32) = 1.12, p = .339$). Measures of the initial and final heading had similar patterns. Figure 4C and 4D show that the absolute initial and final heading angles of the IPN-lesioned rats (initial: $69.5 \pm 5.5^\circ$;

final: $61.7 \pm 5.8^\circ$) deviated further from the refuge than that of Sham rats (initial: $31.7 \pm 2.9^\circ$; final: $22.9 \pm 2.5^\circ$). This was confirmed by significant group effects (initial: $F(1, 16) = 17, p < .001$; final: $F(1, 16) = 14.3, p = .002$). Nevertheless, the ANOVAs did not indicate day (initial: $F(2, 32) = .159, p = .854$; final: $F(2, 32) = .855, p = .435$) or group by day interactions (initial: $F(2, 32) = .226, p = .799$; final: $F(2, 32) = .169, p = .846$). Taken together, the results are consistent with the interpretation that IPN-lesioned rats are unable to use path integration processes to accurately return to the refuge.

Lights-On test

The purpose of the lights-on version of the food carrying task was to assess the ability of rats to navigate using a landmark navigation strategy. First, we examined the measures of search behavior during this version of the task. The left panels in Figure 5 show the search paths of representative rats from IPN-lesioned and Sham groups for the first trial of lights-on training. As observed during lights-off trials, rats from both groups made circuitous searches before finding the food pellets. The groups did not differ in search duration (Table 1) as indicated by a non-significant group effect ($F(1, 17) = .889, p = .359$). Moreover, the ANOVA did not reveal any significant day ($F(2, 34) = .065, p = .937$), or group by day interactions for search duration ($F(2, 34) = .122, p = .885$).

The right panels in Figure 5 show representative return paths made by Sham and IPN-lesioned rats. Note that the return path made by the IPN-lesioned rat was less accurate, less direct, and longer than that of the Sham rat. Indeed, Figure 6A shows that on average IPN-lesioned rats chose the correct refuge ($2.50 \pm .20$) less than Sham animals ($3.81 \pm .08$). A significant group effect for the ANOVA conducted on the number of correct choices confirmed this observation ($F(1, 17) = 19.4, p < .001$). The ANOVA did not indicate a day effect ($F(2, 34) = 1.06, p = .359$), or a group by day interaction ($F(2, 34) = 1.52, p = .234$). Analysis on the duration to return to the refuge after finding the food pellet show that IPN-lesioned rats take longer to find the refuge than Sham rats (Fig. 6B). An ANOVA confirmed a significant group effect ($F(1, 17) = 15.7, p = .001$). Interestingly, the ANOVA revealed significant day ($F(2, 34) = 17.5, p < .001$), and group by day interactions ($F(2, 34) = 3.73, p = .03$). Inspection of Figure 6B shows that the IPN lesioned rats reduced their return latencies from Day 1 ($7.72 \pm .62$ sec) to Day 3 ($4.78 \pm .52$ sec), while the daily return latencies remained the same for Sham rats. Further analysis with planned contrasts confirmed this observation ($p = .01$). A similar pattern of results was obtained for the absolute initial heading (Fig. 6C) and absolute final heading angles (Fig. 6D). The ANOVAs for these measures showed that the initial and final heading angles deviated further from the refuge than that of Sham rats (initial: $F(1, 17) = 36.5, p < .001$; final: $F(1, 17) = 20.3, p < .001$). Additionally, significant day (initial: $F(2, 34) = 7.96, p = .001$; final: $F(2, 34) = 9.35, p = .001$) and group by day effects (initial: $F(2, 34) = 6.67, p = .004$; final: $F(2, 34) = 12.5, p < .001$) were indicated by the ANOVAs. Further analysis with planned contrasts confirmed a significant reduction across days in the initial ($p = .003$) and final ($p < .001$) headings for the lesioned rats. Again, inspection of Figure 6C and 6D clearly show that the IPN lesioned rats reduced their initial and final heading angles from Day 1 (initial: $81.0 \pm 9.4^\circ$; final: $71.4 \pm 13.5^\circ$) to Day 3 (initial: $51.7 \pm 9.5^\circ$; final: $39.0 \pm 8.8^\circ$), while the daily initial and final heading angles remained the same for Sham rats. Taken together, the results demonstrate that an intact IPN is required for accurate landmark navigation. Nonetheless, the impairments were significantly reduced by the final testing day suggesting that some recovery of navigational accuracy is possible after several trials in the landmark navigation task.

Cued platform swimming task

This version of the water maze was used to assess whether the lesioned animals could navigate using a beacon. Two rats from the IPN-lesioned group were excluded from the analysis because they demonstrated poor swimming marked by the absence of movement or floating when placed in the water. These animals were excluded from testing in the subsequent hidden platform version of the task. All other animals in both groups demonstrated that they could accurately navigate toward the cued platform (Fig. 7A). This result was confirmed by a significant block effect ($F(3, 45) = 47.7, p < .001$), as indicated by a repeated measures ANOVA. In addition, the ANOVA showed that the group effect approached significance ($F(1, 15) = 3.79, p = .07$), but was not significant for the group by block interaction ($F(3, 45) = .580, p = .361$). It is possible that the higher mean latencies for the lesioned group during the 2nd and 3rd training block resulted in the near significant group effect (see Fig. 7A). Mean comparisons showed that significant group differences occurred during the 3rd block ($p = .018$), but not for blocks 1, 2, or 4 ($p > .05$), suggesting that the greater swim latencies for lesioned rats during the 3rd block contributed to the near significant group effects. In sum, although IPN lesioned rats had significantly higher latencies during the 3rd training block, they were capable of accurately navigating toward the cued platform by the 4th testing block. Thus, the results suggest that IPN lesions do not interfere with accurate beacon navigation.

Hidden platform swimming task

The hidden platform version of the water maze was used to further evaluate the landmark navigation abilities of the IPN-lesioned animals. Specifically, the task requires that the animals learn the spatial relationship between the fixed room/apparatus cues and the platform location. Figure 7B plots the swim latencies of animals in IPN-lesioned and Sham groups. Overall, rats from both groups showed reduced swim latencies across training, as indicated by a significant block effect ($F(7, 105) = 11.6, p < .001$). Despite the observation that the IPN-lesioned group had numerically greater mean swim latencies (16.4 ± 1.4 sec) than the Sham group (11.0 ± 1.2 sec), the ANOVA did not indicate a significant group effect ($F(1, 15) = 3.51, p = .08$). Similarly, the group by block interaction did not reach statistical significance ($F(7, 105) = .863, p = .539$). It is noteworthy, however, that the IPN-lesioned group had higher swim latencies during blocks 3 to 8 as compared to control rats. Mean comparisons showed that IPN-lesioned rats had significantly greater escape latencies during blocks 6 and 7 ($p = .01, .046$, respectively), but not during blocks 3, 4, and 8 ($p > .05$). A comparison of block 5 latencies approached significance ($p = .059$). In sum, these results suggest that IPN-lesioned animals can learn the position of the platform after 8 blocks of training, but do not reach the same level of performance as Sham rats.

Given the findings described above, it is important to note that several studies have demonstrated that rats can solve the water maze task using a number of spatial cues including the fixed relationship of the platform with the pool walls and the distal room cues (Hamilton et al., 2007;2008;Hoh & Cain, 1997;Morris, 1984). Thus, it is possible that the IPN lesions may have impaired navigation based on a particular information source while sparing other cue sources, resulting in the mild training impairment described above. To determine what spatial information the animals in the present study acquired during training, we further tested the rats in a probe trial 24 hr after the final training block of the hidden platform task. For this probe test, the platform was removed from the pool and the rats were released from the opposite side of the swimming pool and allowed to swim for 60 sec. If during training our rats used the distal room cues to orient and swim to the hidden platform, we would expect that the animals would initially swim toward the platform's previous location and spend a disproportionate amount of time searching at that location during the probe trial. Inspection of the video files revealed that while all 9 of the sham animals swam

directly to the platforms previous location, only 3 out of the 8 lesioned rats swam directly toward the correct location. To assess this difference further, we calculated the percentage of time each rat swam in the four pool quadrants (see Fig. 8A). In general, Sham rats spent more time than IPN-lesioned rats in the quadrant that previously contained the platform (Sham: $58.5 \pm 2.25\%$; IPN: $37.3 \pm 4.88\%$). An ANOVA confirmed this general observation by showing a significant group by quadrant interaction ($F(3, 45) = 8.62, p < .001$), with a significant group difference in the target quadrant ($p = .01$). Inspection of Figure 8A suggests that although the IPN lesioned rats showed poorer retention for the platforms previous location, the lesioned animals spent more time swimming in the correct quadrant than in other quadrants. Confirming this observation, an ANOVA on the quadrant times for lesioned rats showed a significant effect of quadrant ($F(3, 21) = 4.27, p = .02$). On average, lesioned rats spent a greater percentage of time in the target quadrant ($37.3 \pm 4.88\%$) than in the other quadrants (average time in other quadrants: $20.6 \pm 1.6\%$). A mean comparison demonstrated that this difference reached statistical significance ($p = .04$). Taken together, these data suggest that the IPN-lesioned rats showed some retention for the platform's previous location, however, their performance did not reach the level of the Sham rats.

We further assessed the ability of the animals to use the pool walls as a spatial cue by dividing the maze into three annuli (see inset of Fig. 8B). We reasoned that if the rats were capable of using the pool walls as a cue, we would observe a greater amount of search time in the annulus marking the platform position relative to the wall (the middle annulus). Inspection of the video files showed that Sham rats generally searched in the correct quadrant and annulus, while IPN lesioned rats generally searched in the correct middle annulus but not in the correct quadrant. That is, lesioned rats generally swam along the middle annulus. As shown in Figure 8B, search preference for the middle annulus was greater than the other annuli for both groups of animals, and did not vary between the IPN-lesioned and Sham groups (mean for middle annulus, IPN: $47.5 \pm 5.2\%$; Sham: $46.7 \pm 3.69\%$). Confirming this observation, an ANOVA on the percentage of swim time in each pool annuli did not reveal group ($F(1, 15) = .882, p = .362$), or group by annuli effects ($F(2, 30) = .078, p = .925$), but did reveal a significant annuli effect ($F(2, 30) = 12.3, p < .001$). This latter significant result reflects the search preference of both groups of animals for the middle annulus over the other two annuli. Considering this finding with that of the quadrant analysis, our results collectively demonstrate that IPN lesioned rats were capable of learning the distance from the swimming pool walls to the platform, but were impaired at using the distal visual cues to disambiguate the correct quadrant for a focused search.

Discussion

The IPN has been linked to a variety of behaviors including homeostasis, olfaction, stress, sleep, nociception, and avoidance learning (reviewed in Klemm, 2004; Morley, 1986). This implication in a wide range of behaviors may stem from the fact that it contains several morphologically distinct subnuclei and discrete subregional distributions of neurotransmitters (Groenewegen et al., 1986). At present, it is unknown whether the IPN has role in navigation. Thus, the purpose of the present study was to determine whether the IPN is involved in three forms of navigation: beacon, landmark, and path integration. IPN lesions were produced by infusing the neurotoxin NMDA into the IPN at six rostral-caudal levels. After the rats recovered from the surgery, they were tested in a food-carrying paradigm that they had acquired before surgery and then a standard cued platform and hidden platform Morris water maze procedure that they had to acquire post-surgery (Frohardt et al., 2006; Morris et al., 1982; Whishaw et al., 2001). Our findings demonstrate that IPN lesions impair accurate navigation in tasks evaluating landmark navigation and path integration, but not in tasks assessing beacon navigation. Impairments in landmark navigation were milder than path integration deficits, as lesioned rats showed some recovery of navigational accuracy

across testing trials. In sum, these results suggest that the IPN serves a fundamental role in navigation.

IPN lesions and path integration

The path integration task given to rats with IPN damage was similar to that given previously to rats with lesions of place cell (hippocampus), grid cell (entorhinal cortex), and HD cell circuitry (anterior thalamus and dorsal tegmental nuclei) (Frohardt et al., 2006; Maaswinkel et al., 1999; Parron & Save, 2004; Save et al., 2001; Whishaw et al., 2001). Sham and IPN lesioned rats were trained in the food-carrying paradigm in which they searched for food pellets in an open-field, and directly returned to their home refuge after finding the food. We evaluated the animal's ability to use self-movement cues to accurately return to the home refuge by turning the room lights off and monitoring their behavior under infrared light, a wavelength that rats cannot see (Neitz & Jacobs, 1986). Olfactory and auditory cues were displaced by cleaning the apparatus and refuge between foraging trials and playing white noise from a radio above the table. To further ensure that room cues were not used, the home refuge was moved to a different doorway at the beginning of each trial. Previous work has shown that similar testing conditions promote the use of path integration rather than landmark or beacon based navigational strategies (Frohardt et al., 2006; Maaswinkel et al., 1999). Under these conditions, sham rats were able to return accurately to the home refuge, suggesting that they were capable of integrating self-movement information to determine a direct return to the refuge (Frohardt et al., 2006; Maaswinkel et al., 1999; Parron & Save, 2004; Whishaw et al., 2001; Whishaw & Tomie 1997). In contrast, rats with lesions of the IPN were less accurate and less direct when returning to the refuge under infrared conditions. This result suggests that the IPN lesions interfered with accurate path integration.

Navigation based on path integration likely involves several computational and behavioral characteristics including: 1) the establishment of an initial starting point or home base, 2) the computation of a current orientation (direction, location, and distance) relative to a starting point, and 3) the computation of a direct trajectory back to the starting point or some other goal. This last computational step requires that the animal compute the angular distance it must turn in order to embark on the correct trajectory. These steps are often described in terms of a vector-based system in which, 1) the path integration vector is reset or nulled before the animal leaves the home base, 2) the vector pointing to the home base is updated by integrating speed, distance, and directional changes while the animal moves through its environment, and 3) the vector is decoded so as to select a proper homing direction for return (Etienne and Jeffery, 2004). However path integration is defined, it is unlikely that IPN lesions interfered with the establishment of the refuge as a reference point because animals from both groups spent most of their time in the refuge during food-carrying trials and persistently searched and eventually located the refuge after finding the food pellet. Thus, it is likely that the IPN influences one or more of the other subsequent steps.

One possibility is that the IPN contributes to path integration through its dense inputs to the HD cell circuit via the dorsal tegmental nuclei. Indeed, researchers have proposed that the cells and pathways comprising the HD cell circuit are used to compute the directional component of path integration (Burgess et al., 2007; McNaughton et al., 1996; Redish, 1999). Consistent with this view is a recent report from our laboratory in which the activity of anterior thalamic HD cells was monitored in animals that had large neurotoxic or electrolytic lesions of the IPN (Clark et al., 2008). HD cells were recorded during conditions that required the integration of self-movement cues to maintain their preferred firing directions. In these path integration-dependent tests, HD cells in intact animals maintained their preferred firing direction, whereas HD cells in IPN lesioned animals failed to

accurately maintain their preferred directions. Thus, directional path integration was impaired in rats with IPN lesions.

We have suggested that the IPN conveys motor information to the HD cell circuit because of its intimate connectivity with the habenula which receives motor information from basal ganglia output structures (Contestabile & Flumerfelt, 1981; Groenewegen et al., 1986; van der Kooy & Carter, 1981), and the finding that the IPN contains cells sensitive to the animal's movement speed (Sharp et al., 2006). Thus, it is possible that the path integration impairments observed in the present study stem from depriving the HD cell circuit of motor based self-movement cues. The IPN may alternatively influence path integration processes via its well documented projections to the dentate and CA3 subfields of the hippocampus (Baisden et al., 1979; Groenewegen et al., 1986; Montone et al., 1988; Segal, 1975; Shibata & Suzuki, 1984), which along with CA1 area have been linked to path integration processing (Golob & Taube, 1999; McNaughton et al., 1996; Whishaw et al., 2001). Future work investigating hippocampal place cell activity in animals with IPN lesions may shed light on this issue. Interestingly, anatomical evidence indicates that the caudal subnuclei of the IPN projects to the hippocampus, whereas the rostral subnuclei of the IPN project most prominently to the DTN (Groenewegen et al., 1986; Hemminger & Moore, 1984; Liu et al., 1984). It will be important for future experiments to examine this distinct anatomical relationship, as well as the functional role served by the connections between the different IPN subnuclei.

IPN lesions and landmark navigation

Rats from sham and IPN lesioned groups were given two tests to evaluate their landmark navigation abilities. First, rats were tested in the food-carrying task with the room illuminated and the refuge left in a consistent location. Like the lights-off version of the food-carry task, olfactory and auditory cues were obscured by washing the open-field between trials and playing white noise from a radio above the table. Under these testing conditions, sham rats returned to the refuge accurately and directly, while IPN lesioned rats were less accurate, less direct, and took longer to find the refuge location. Although this finding suggests that IPN lesions interfere with the ability to navigate based on visual landmarks, it is possible that the rats elected to use path integration rather than landmark based strategies when the room lights were turned on. We addressed this possibility by testing the rats in a standard hidden platform version of the Morris water maze (Morris, 1984; Morris et al., 1982). In this task, the rats were pseudorandomly placed at one of four start points at the periphery of the swimming pool and were required to swim and climb a platform that was submerged just below the surface of cool opaque water. To reliably locate the hidden platform, the task requires that the rats learn the relationship between the visual room cues and the platform location. By the end of testing, sham rats readily localized the position of the hidden platform and showed retention for the platform position in a probe test. In contrast, IPN lesioned rats had longer latencies to find the hidden platform and showed less retention for the platform location during the probe test. Collectively, the results from both tests suggest that IPN lesions interfere with accurate landmark navigation.

Conceptually, learning can be divided between task acquisition and accurate performance. For spatial tasks involving landmark navigation this first entails learning the procedural aspects of the task, followed by learning the relationship between the goal and ambient room cues (Bannerman et al., 1995; Saucier & Cain, 1995). For instance, in the hidden platform variant of the Morris water maze, the rat must learn how to swim, learn that it cannot escape by searching at the sides of the pool, learn that escape is possible by obtaining purchase on the pool platform, and learn to wait on the platform until it is removed and placed back in the home cage. After the rat is familiar with these procedural demands, it can then learn the location of the platform in relation to the room/apparatus cues. Because interpretations of

landmark navigation deficits are confounded if procedural and landmark learning are not distinguished, we elected to test sham and lesioned rats in a cued (beacon navigation) variant of the Morris water task before testing their navigation in the hidden (landmark navigation) variant. Thus, the cued platform task not only evaluated beacon navigation, but was also used to ensure that the animals could learn the procedural demands of the Morris water task before assessing landmark navigation. As a consequence, two lesioned rats were removed from the experiment because they displayed marked swimming impairments characterized by long bouts of floating and the absence of movement. For the remaining rats, it was clear that IPN lesions spared the ability to accurately swim to a cued platform, but impaired navigation to the hidden platform, suggesting that the lesions impaired landmark learning rather than the acquisition of the task procedures.

Like the water maze, the food-carrying task requires that animals learn to search and find a large food-pellet and then carry it to the refuge location. It is unlikely that IPN lesions interfered with the performance of the procedural elements of the task. First, animals in both groups were pre-surgically familiarized with both lights-off and light-on testing conditions. Furthermore, after recovery from surgery, animals from both groups displayed reliable food-carrying by exiting the refuge rapidly (~3-5 sec), searching the open-field for food, and carrying the food-pellet to a doorway. When an incorrect doorway was selected, animals from both groups visited other doorways until they found the correct one. Thus, the deficits observed in IPN lesioned rats during lights-on and lights-off testing are more likely to involve impairments of landmark navigation and path integration, respectively.

While the results of the present study suggest that the IPN has a role in landmark navigation, it is unclear whether the structure is differentially involved in the acquisition, retention, or retrieval of landmark information. Lesions of the IPN clearly disrupted the retention of the pre-surgically acquired lights-on task, but had a weaker effect on new learning. For example, IPN lesioned rats showed significant improvements in landmark navigation across testing days in the food-carrying task and the hidden platform task. During the water maze probe trials, lesioned rats searched in the correct pool quadrant more than the other quadrants, although not nearly as accurately as sham rats. In addition, we noted that many of the lesioned rats swam in circles at a fixed distance from the pool wall such that their swim paths crossed the platforms previous position. This finding indicates that although the lesioned rats were poorer than shams at using the distal visual cues to focus their search, the rats were capable of estimating the distance of the platform position from the pool wall. Similar observations have been made in rats with hippocampal and entorhinal cortex lesions (Morris et al., 1990; Schenk & Morris, 1985). These results suggest that while their landmark navigational abilities were generally impaired, other strategies and sources of information are available through pathways independent of the IPN.

The results of the landmark navigation tests demonstrate that IPN lesions produce mild impairments in landmark navigation, despite the fact that the IPN is several synapses removed from the cortical circuitry often linked to the processing of landmark information. Given the fact that IPN lesions severely impair path integration, perhaps by removing the normal flow of motor information to limbic brain areas, one possible explanation for the landmark navigation deficits is that a stable spatial framework based on path integration information is required for landmark cue learning (Alyan & Jander, 1994; McNaughton et al., 1996). In other words, landmark learning may occur in relation to path integration, and not independent of it. Although work by some investigators supports this view (Dudchenko et al., 1997; Gibson et al., 2002; Knierim et al., 1995; Martin et al., 1997), it is still currently unclear whether path integration and landmark navigation is subserved by independent neural structures or whether they are mediated by a common neural substrate. Future work should be directed at this issue.

Conclusion

In summary, IPN lesions impair accurate navigation in tasks evaluating landmark navigation and path integration, but not in tasks assessing beacon navigation. Landmark navigation impairments were generally mild, as lesioned rats showed marked improvements in navigation accuracy across testing days in both the food-carrying and water maze spatial tasks. The IPN and its related circuitry comprise a major link between limbic forebrain, striatum, and brainstem structures (Klemm, 2004; Lecourtier & Kelley, 2007; Sutherland, 1982). Future work should be directed at elucidating the precise role this circuitry has on spatial behavior and in learning and memory in general.

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Figure 1.

(A.) The food-carrying apparatus. The test apparatus consisted of a grey rotatable circular table with surrounding walls that contained eight potential refuge locations hidden behind black curtains. A fixed platform was mounted in the center of apparatus. Notice that a floor-to-ceiling black curtain surrounds the maze apparatus and a white floor-to-ceiling curtain hangs ~135° opposite to the refuge. (B.) An overhead view of the food carrying apparatus. At the center of the apparatus is an example sequence in which the central food dishes were baited during a single testing day. The diagram also illustrated the four refuge locations (A, B, C, or D) used during lights-off testing. Refuge location A was used throughout lights-on testing.

Figure 2.

(A.) Select plates from Paxinos and Watson (1998) showing the rostral-caudal extent of the largest (grey) and smallest (black) IPN lesions at relative coordinates from bregma. (B.) An enlarged view of the boxed area in A in a representative section from a Sham rat. (C.) A representative section from a rat with neurotoxic damage in the IPN. Note the glial scar that passes through the IPN. The black arrows point to damage extending into structures dorsal to the IPN (paranigral nucleus and interfascicular nucleus). (D.) and (E.) show close-up images of the sections from a representative control and IPN lesioned rat at the location indicated by the black box in sections B and C, respectively. Note the absence of neurons and the greater number of glial cells in the section from the IPN lesioned rat (E.).

Figure 3.

Representative paths taken by Sham (top) and IPN-lesioned (bottom) rats on their search (left panels) and return (right panels) trips during the first trial of lights-off testing. The black box represents the refuge location and the open circle represents the food pellet location. Note the increased path length and incorrect initial directional heading on the return trip for the IPN-lesioned rat.

Figure 4.

Return trip dependent measures for Sham (open circles) and IPN-Lesioned (closed circles) for each day of lights-off testing. **(A.)** Number of correct refuge choices (mean $\pm$ SEM). **(B.)** The time taken to return to the refuge (mean $\pm$ SEM). **(C.)** The initial (mean $\pm$ SEM) and **(D.)** final heading angle (mean $\pm$ SEM) from the refuge location. A perfect return heading to the refuge would be 0° .

Figure 5.

Representative paths taken by Sham (top) and IPN-lesioned (bottom) rats on their search (left panels) and return (right panels) trips during the first trial of lights-on testing. The black box represents the refuge location and the open circle represents the food pellet location. Note the increased path length and incorrect initial directional heading on the return trip for the IPN-lesioned rat.

Figure 6. Return trip dependent measures for Sham (open circles) and IPN-Lesioned (closed circles) rats for each day of lights-on testing. (A.) Number of correct refuge choices (mean ± SEM). (B.) The time taken to return to the refuge (mean ± SEM). (C.) The initial (mean ± SEM) and (D.) final heading angle (mean ± SEM) from the refuge location. A perfect return heading to the refuge would be 0°.

Figure 7.

(A.) Latency (mean $\pm$ SEM) for the Sham and IPN-lesioned rats to navigate to the cued platform during the four training blocks (4 trials each). (B.) Latency (mean $\pm$ SEM) for the Sham and IPN-lesioned rats to navigate to the hidden uncued platform during the eight training blocks (4 trials each).

Figure 8.

(**A.**) Bar graph showing the percentage of time (mean $\pm$ SEM) that animals spent swimming in the four swimming pool quadrants during the probe trial. The black bars indicate the quadrant that contained the platform during training (see inset). (**B.**) Bar graph showing the percentage of time (mean $\pm$ SEM) that animals spent swimming in the three pool annuli. Black bars indicate the annulus that contained the platform during training (see inset). The white circle in the inset indicates the platform location.

Table 1Average ($\pm$ SEM) Search Duration

Group	Condition	Day	Search Duration (sec)
Sham	Infrared	One	11.84 $\pm$ 1.88
		Two	13.03 $\pm$ 2.88
		Three	15.88 $\pm$ 3.04
IPN-Lesion	Infrared	One	15.93 $\pm$ 3.36
		Two	12.53 $\pm$ 1.47
		Three	12.10 $\pm$ 1.55
Sham	Lights-On	One	10.94 $\pm$ 1.63
		Two	12.28 $\pm$ 3.07
		Three	11.19 $\pm$ 2.43
IPN-Lesion	Lights-On	One	9.58 $\pm$ 1.72
		Two	9.25 $\pm$ 1.26
		Three	9.23 $\pm$ 2.24