Lesions of the Head Direction Cell System Impair Direction Discrimination

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Abstract

Previous results suggest that directional information from the head direction cell circuit may inform hippocampal place cell firing when an animal is confronted with visually identical environments. To investigate whether such information might also be essential for spatial behavior, we tested adult, male Lister Hooded rats that had received either bilateral lateral mammillary nuclei (LMN) lesions or sham lesions on a four-way, conditional odor-location discrimintion in compartments arranged at 60^{o} to one another. We found that significantly fewer rats in the LMN lesion group were able to learn the task compared to the Sham group. We also found that the extent of the behavioral impairment was highly correlated with the degree of tissue loss in the LMN resulting from the lesion. Animals with LMN lesions were also impaired in a non-matching to sample task in a T-maze, although the extent of impairment was not sensitive to the extent of the lesion. Performance in the odor-location and T-maze tasks was not affected by tissue loss in the medial mammillary nuclei. Together, these results indicate that the LMN, a key node in the head direction circuit, is critical for solving a spatial task that requires a directional discrimination.

Key words: lateral mammillary nuclei, head direction cells, spatial cognition, associative memory, T-maze, medial mammillary nuclei, odor discrimination, context discrimination

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The head direction cell system is an interconnected set of brain regions that contain head direction cells. Individual head direction cells fire robustly when an animal, such as a rat or mouse, faces a specific direction within its environment, but are otherwise silent (Taube, 2007). Despite the robust firing of head direction cells and their presence in several brain regions, the exact function of these cells has yet to be established.

The head direction signal is generated in the reciprocal connections between the dorsal tegmental and lateral mammillary nuclei (LMN) (Clark and Taube, 2012; Yoder et al., 2015). The HD signal is then propagated along a pathway that encompasses the anterior dorsal nucleus of the thalamus, and postsubiculum, and the medial entorhinal cortex (Yoder et al., 2015). In support of this, lesions of the LMN disrupt the signal in upstream structures such as the anterior dorsal thalamus (Blair et al, 1998; 1999), subiculum, postsubiculum, and medial entorhinal cortex (Sharp & Koester 2008a).

One approach to assessing the function of the head direction (HD) cell system is to lesion portions of this circuit in rodents, and to assess the ensuing impact on spatial behavior. This approach has yielded mixed results. Lesions of the LMN do not tend to impair alternation in a T-maze (Vann, 2005, 2011). However, in a version of the T-maze task where sample and choice phases were conducted on different mazes, rats with LMN lesions exhibited an impairment in performance relative to control animals. This finding suggests that head direction information becomes more important in tasks in which making correct choices relies more heavily on directional information, in this case due to the absence of intra-maze cues and the presence of potentially misleading room cues (Vann, 2011). In tasks where distal room cues are both visual and salient, lesions of the LMN have little effect. For example, in a task where animals were

required to learn the location of a sand cup containing a food reward relative to a salient landmark, LMN lesions had no effect on performance (Harland et al, 2015). Similarly, when room cues were present, animals with LMN lesions were not impaired relative to controls on a radial arm maze task (Vann, 2018). Transient impairments have been observed following LMN lesions in a Morris water maze task that required the use of the geometry of a rectangular pool to locate a hidden platform whose location changed with respect to room cues but not to pool geometry (Vann, 2011; see also Harland et al., 2015). A potential difficulty in assessing this literature is that spatial tasks, such as those used in the aforementioned studies, can be solved using different strategies. Only some of these strategies may require the head direction cell system.

In the current study, we approached this question from a different perspective. Previous work has shown that in a maze apparatus comprising a series of visually identical compartments arranged parallel to one another, hippocampal place cells (neurons that fire in specific locations) are active in equivalent locations in each compartment - a phenomenon termed place field repetition (Derdikman et al., 2009; Grieves et al., 2016; Spiers et al., 2015). However, place cells remap between compartments when these are arranged in different directions to one another, even in the absence of extramaze cues (Fuhs et al, 2005; Grieves et al., 2016). Thus, it was hypothesised that the rats' sense of direction could inform place cell firing. Although place cell firing remains intact following lesions of the LMN (Sharp and Koester, 2008a), rats with lesions to the LMN exhibit hippocampal place field repetition across radially arranged compartments (Harland et al., 2017). Thus, the head direction cell system may be essential for the discrimination of compartments that face different directions.

Consistent with this hypothesis, in a behavioral task in which rats had to associate a particular odor with a particular compartment in order to locate a food reward, animals were more successful at learning the association when the compartments were arranged radially rather than in parallel (Grieves et al., 2016). This observation implies that rats use directional information to disambiguate visually similar

environments. What has yet to be shown, however, is whether disruption of the head direction cell system impairs such directional discriminations.

The aim of the present study was thus to investigate the role of HD cells in the LMN in behavioral disambiguation of visually similar environments. We hypothesized that head direction information is required to discriminate between visually identical compartments arranged at a different direction to one another. Based on this hypothesis, we predicted that animals with lesions to the LMN would show impaired performance in the four-way, odor-location conditional discrimination in which the animals are required to associate a specific odor with a specific maze compartment.

Method

Animals

Experimental subjects were 22 adult male Lister hooded rats (Charles Rivers Laboratories, UK) weighing 330-520g at the start of the experiment. Animals were housed in cages (2-4 rats per cage) on a 12-hour light-dark cycle with ad libitum access to water. During the period of behavioral experiments, animals were mildly food restricted so that they maintained ~90% (and no less than 85%) of their free-feeding body weight. All behavioral testing was conducted during the light phase. All procedures were carried out in accordance with the UK Animals (Scientific Procedures) Act (1986) and the European Communities Council Directive of November 24, 1986 (86/609/EEC). All regulated procedures were carried out under a UK Home Office Project Licence by researchers with UK Home Office Personal Licences. Experimental protocols were pre-approved by the University of Edinburgh Animal Welfare and Ethical Review Board (AWERB).

Surgery. Animals were pseudo randomly assigned to either the sham (n = 9) or lesion (n=13) group, such that animal weights were matched between groups. All animals were anaesthetised with isoflurane (Vetflurane, Virbac, UK) and placed in a stereotaxic frame. An incision was made in the scalp and the skin was retracted to expose the skull. A 1.8mm trephine drill bit (Fine Science Tools, Germany) was used to drill through the skull at the appropriate coordinates. Animals in the lesion group received bilateral ibotenic acid lesions in the LMN. Lesion injections consisted of 0.45µl 10mg/ml ibotenic acid (Tocris Bioscience, UK) per hemisphere, delivered via a blunt 1µl Hamilton syringe angled at 10^{o} posterior (tip pointing towards the tail of the animal). The injection was aimed at the following coordinates relative to bregma: -4.5mm anterior-posterior (AP), ±1.0mm medial-lateral (ML), -9.2mm dorsoventral (measured from dura). Injection at these coordinates was achieved by measuring the bregma-lambda AP difference, and setting the point of entry into the brain as bregma - 0.375 x this difference. Similarly, ML coordinates were measured as ±1.0mm from the midpoint between lambda and bregma in the ML plane. Injections were given over the course of 5 min with the needle left in situ for a further 5 min to allow for diffusion of the ibotenic acid before retracting. For animals in the Sham group, an empty needle was lowered to the same coordinates but no material was injected. After completion of lesions (or sham injections), burr holes were plugged with an absorbable haemostatic gelatin sponge (Spongostan, Ethicon, USA), and the skin was cleaned and sutured. Animals were given subcutaneous injections of 0.08ml/kg carprofen (Rimadyl, Pfizer, UK) at the start of surgery for pain relief and injections of glucose 5% in saline (Baxter, UK) during surgery for rehydration. After surgery, animals were closely monitored and allowed to recover for seven days before food restriction and testing began. All behavioral testing and scoring was conducted with the experimenters blind to the group allocation of the rats.

Apparatus

Odor shaping. For the initial odor shaping training, 40cm x 60cm x 50cm wooden box was used. The box was placed in the experimental room on wooden stools that elevated it 80cm above the ground.

Four-compartment apparatus. Experiments took place in a four-compartment environment in which the compartments were arranged at 60^{o} to one another and were joined by a central corridor (Figure 1). Each compartment was 40.4 cm long, 35.5 cm wide, 30 cm tall, and with a 15.5 cm doorway cut into one of the short sides. The corridor was of width 19 cm. Both the compartments and the corridor were constructed from plywood and were painted blue. Barriers could be inserted into the compartments to block the doorways when desired. Four Nalgene pots containing scented sand were placed against the back wall of each compartment and secured with an adhesive reclosable fastener (Dual Lock, 3M, UK). Sand-odor mixtures consisted of 0.5g of powdered odor substrate and 2g of chocolate cereal dust per 100g of sand. The chocolate dust was added to odorised sand pots in order to mask the odor of the food reward. The compartments and corridor were placed on wooden stools to elevate them 80cm from the ground. The apparatus was located in a black curtained enclosure with an opaque white ceiling and lit with a bulb situated in the centre of the ceiling. During trials the curtains were kept closed at all times and white noise was played in order to eliminate local and distal visual and auditory cues, so that the structure of the maze itself was the sole directional cue available to the rats.

T-maze. Experiments took place in a T-maze which was constructed from three separate arms. Each arm was 45.5cm long, 12.0cm wide, and 16cm in height. The arms had wooden floors, painted blue, and clear Perspex walls. A ceramic pot was placed at the end of each of the reward arms to obscure the reward from view. An opaque, wooden barrier was placed in the start arm to contain the rat before the start of a trial. The maze was placed on wooden stools to elevate it 80cm from the ground. Laboratory room cues were visible from the maze.

Procedure

Odor shaping. Animals were first trained to dig in pots of unscented sand until they reliably retrieved a food reward (½ Weetos chocolate hoops cereal, Weetabix, UK) buried 2/3rds of the way down a pot. Once they did this, the rats were shaped to discriminate odors by presenting them with two scented

sand pots next to one another. One pot was scented with coffee and one with fennel. Only one scent was rewarded per rat. Rats were allowed to dig in both pots until the reward was retrieved, after which the animal was confined to the opposite end of the box behind an opaque barrier while the reward was replenished. Animals received 10 such trials per day until the criterion of 9/10 correct first choices for two days in a row was reached. A choice was defined as an animal displacing the sand using one or both paws. The side of the box where the rewarded odor was located was counterbalanced across trials to prevent animals learning the identity of a rewarded odor by its location. The identity of the rewarded odor was also counterbalanced across animal groups.

Odor-location conditional discrimination training. Animals were trained in three stages on the four-way, odor-location conditional discrimination (Figure 1). In this task, each of the four compartments of the apparatus contained four pots of sand, each scented with a different odor (basil, coriander, cumin or rosemary). odorised sand mixtures consisted of 0.5g powdered odor substrate and 2g chocolate cereal dust per 100g of sand. The same four odors were presented in the same relative locations in each compartment, but the identity of the rewarded odor was different in each compartment. Thus, in order for the rat to obtain the reward, it had to be able to recognise which compartment it was in, and also recall the odor that was rewarded in that specific compartment. Due to the difficulty of the task, the animals were trained in stages. In the first stage, only two compartments were available to the animal, and each compartment contained two odorised sand pots, and two pots filled with unscented sand. At the start of each trial, the animal was placed in the corridor with all four compartments blocked by doors. The door to one compartment was then lifted, and the rat was allowed to dig in all pots until it found the reward. The rat was then returned to the corridor and the reward was replaced. Rats were given six such trials in each compartment (12 trials overall for the two compartments in a pseudorandom order) per day for three days. From day four onwards trials continued but the animal was removed from a compartment after its first choice dig, regardless of whether the reward was retrieved or not. Rats were tested on the two-

compartment stage until they met the criterion of 5/6 correct choices in each compartment for two consecutive days. Animals not reaching this criterion after 40 days did not progress to the second testing stage. In the second stage, three compartments were available to the animal, and one of the pots that had previously contained unscented sand was now odorised. That new odor became the rewarded odor in the newly available compartment. Rats were again given six trials per compartment per day (18 trials overall for the three compartments) until they had reached the criterion of 5/6 correct choices in each compartment for two consecutive days. Animals not reaching criterion after 30 days did not progress to the final testing stage. In the final stage, all four compartments were used, and the remaining unscented sand pot was odorised in each compartment, with that odor becoming the rewarded odor in the fourth compartment. Again, rats were given six trials per compartment per day (24 trials overall for the four compartments) until they had reached the criterion of 5/6 correct choices in each compartment over two consecutive days. Animals not reaching 5/6 correct choices on or after 20 days were removed from the experiment. One animal from the Sham Group achieved 5/6 correct choices on the 20th session, and was given an additional training session to see if it could do so on two consecutive days. At all stages the maze was cleaned in between animals, and sand pots were replenished to ensure consistent levels of sand. At the end of each day the compartments were swapped such that the discrimination could not be solved using subtle distinctions in the appearance of each compartment. In the event that an animal made 5/6 correct choices on the 'cutoff' day (day 40 for 2-compartment stage, day 30 for 3-compartment stage, day 20 for 4-compartment stage) they also underwent a session on the following day. If they also made 5/6 correct choices on that day they were judged to have reached criterion for advancement in the task.

Non-rewarded probe days. Animals completing the four-compartment stage of the task then progressed to three days of probe trials. This stage was identical to the four-compartment stage of the task, with the exception that no reward was present in two of the six trials for each compartment.

Counterbalancing. In the first stage of the task, either the first and third compartments or the second and fourth compartments were available. In the second stage of the task, the compartment situated between the compartments available in stage one became available. The identity of the available compartment was counterbalanced across animals and between groups. The identity of the rewarded odor in each compartment was varied across animals and counterbalanced between groups. The order in which animals visited each compartment was determined pseudorandomly, such that animals never visited the same compartment more than twice in a row, but visited each compartment twice in a row at least once per day. Probe trials were generated using a random number generator, with the caveat that no more than two unrewarded trials occurred consecutively.

T-maze task pre-training. Animals were habituated to the maze by being placed singly in the apparatus and being allowed to forage for randomly scattered food rewards for 5 minutes. The following day, food rewards were placed only in the ceramic pots at the end of each maze arm, and not replenished until the animal had visited both arms.

Delayed spatial alternation on a T-maze. Each trial consisted of a sample phase and a choice phase. In the sample phase, the animal was placed into the start arm behind an opaque barrier and one arm of the maze was blocked. The barrier was opened, and the animal was allowed to enter the unblocked arm and retrieve a reward from the pot at the end of that arm. The animal was then placed back in the start arm behind the opaque barrier for a 5-second delay period. In the choice phase, both maze arms were open, and the reward was placed in the pot at the end of the arm that had been unavailable during the sample phase. An animal was considered to have made a choice when when all four paws had crossed the threshold of a given arm. Animals were given eight trials per session, with an inter-trial interval of approximately 3 min. The maze was cleaned between each trial and each animal. Sessions continued for eight days. The identity of the sample arm was varied pseudorandomly across trials, such that each arm was available for 4/8 trials per session and did not appear more than twice in a row.

Histology. At the end of experiments, animals were terminally anaesthetised then perfused with phosphate-buffered saline followed by 4% formalin. Brains were extracted and incubated in 4% formalin for at least 48 h before being immersed in 30% sucrose (Sigma, UK) in PBS for 72 h. Brains were then frozen and 40 µm coronal sections were cut at the region of the LMN. The sections were stained with Nissl stain (0.1% cresyl violet solution, Sigma, UK) and coverslipped.

Quantification and statistical analyses

Behavior. For the conditional odor-location task, the experimenters recorded the number of correct digs per session, and for the T-maze, the number of correct arm entries was recorded. Statistical tests were performed using SPSS Statistics 22 (IBM). Associations between dichotomous variables were tested using Fisher's exact tests. Independent samples t-tests were used for simple comparisons between the Sham and LMNx groups. Comparisons between Sham and LMNx groups that also involved repeated measures were performed using two-way mixed ANOVAs. Linear regression was used to explore predictive relationships between lesions and task performance.

Histology. Images of the LMN in Nissl-stained sections were obtained using a microscope (Leica DMRB, Germany) using 2.5x objectives and a QICAM camera (QImaging, Canada) and ImagePro software (Media Cybernetics, USA). In both sham and lesioned groups, the area of the LMN was quantified using an outline tool in ImageJ software. From these measurements, four equivalent sections from each brain were chosen to represent the LMN. The sum of the area occupied by the LMN in both hemispheres on these four sections was calculated. For the Sham group, a value for mean LMN area was then calculated. Values for individual brains in the LMN lesioned group (LMNx) were then compared to this value to calculate the percentage of spared LMN tissue. As previous studies have identified incidental MMN damage as a potential confound in tasks tasting the effect of LMN lesions on spatial memory (Vann, 2010), the procedure was then repeated to calculate the percentage of spared MMN tissue.

Results

Histology

One rat from the LMNx group was excluded from the analysis due to the observation of an enlarged third ventricle when examining histological sections. An additional rat from the LMNx group was killed due to complications unrelated to the experiments after completing the odor-location task, and therefore did not participate in the T-maze task. Hence, the final sample sizes for the two tasks were odor-location task: sham n=9, LMNx n=12; T-maze task: sham n=9, LMNx n=11.

The LMNx group had a mean percentage tissue loss of 67.66% (SD 24.27%, SEM 7.01%) in the LMN, and a mean percentage tissue loss of 16.48% (SD 15.22%, SEM 4.29%) in the MMN (Figures 2A,B). As can be seen from Figure 2B, there was variability in LMN lesion extent, and in the extent of incidental MMN damage. However, there was no significant correlation between the percentage tissue loss observed in LMN and that observed in the MMN (Pearson's correlation $r(10)=0.024$, $p=0.942$), indicating that the degree of tissue loss in MMN cannot be explained by that in LMN (and vice versa).

Odor shaping task

We first tested whether rats in both groups were able to perform the simple odor discrimination that is the basis for the four-way odor-location task. Animals were presented with two sand pots, one scented with coffee and one with fennel, and had to learn that a food reward was buried in the pot scented with one odor but not the other. Animals in the Sham group took an average of 7.56 ± 1.14 days (mean ± SEM) to reach criterion, whereas animals in the LMNx group took 4.50 ± 0.42 days. All animals in both groups successfully reached criterion, however animals in the LMNx group took significantly fewer days to reach criterion compared to the Sham group ($t_{(19)}=2.79$, $p=0.012$, independent samples t-test). Hence, LMN lesions did not impair the ability of animals to discriminate odors or learn to associate a specific odor with a reward.

Odor-location conditional discrimination

To assess the effect of LMN lesions on disambiguation of visually identical environments, animals were trained on the four-way odor-location conditional discrimination task in three stages: two-compartment, three-compartment and four-compartment. While all animals in both groups completed the two-compartment stage, only 7/12 rats in the LMNx group reached criterion at the three-compartment stage (p=0.045, Fisher's exact test) and only 6/12 reached criterion at the four-compartment stage (p=0.019, Fisher's exact test). All rats (9/9) in the Sham group completed the three- and four-compartment stages (Figure 3). Overall, while all rats in the Sham group successfully learned the task (i.e., they reached criterion at all stages of training), only half of the animals with LMN lesions did so.

The number of sessions required for animals to reach criterion at each stage is shown in the cumulative frequency graphs of Figure 4A. As can be seen from these graphs, the Sham group typically had a greater number of animals at criterion on any given session across the three stages, and a smaller proportion of animals in the LMNx group reached criterion in the three- and four-compartment stages compared to the Sham group.

The data for number of sessions to criterion are shown in Figure 4B. Animals in the LMNx group took significantly more sessions to reach criterion compared to animals in the Sham group at the two-compartment stage (t(19)=2.199, p=0.041), but not at the three-compartment (t(19)=1.584, p=0.130) or four-compartment (t(14)=0.980, p=0.344) stages. It should be noted, however, that the number of sessions to criterion was capped at each stage, and five animals in the LMNx reached the limit (30 days) in the three-compartment stage, as did one animal in the four-compartment stage (while no animals in the Sham group did so). Moreover, only the animals that reached criterion at the three-compartment stage were included in the trials to criterion measure for the four-compartment stage.

Probe trials. After reaching criterion on the four-compartment stage of the task, animals then moved on to probe sessions. During each of the three probe sessions, the food reward was removed from the target pot in two out of the six trials in each compartment. The purpose of these was to test whether rats were using the scent of the buried food reward to choose the correct pot. Results are shown in Figure 5. A two-way mixed ANOVA found no statistically significant interaction between trial type and group ($F_{(1,14)}$=0.093, p=0.76), and no significant main effect of trial type ($F_{(1,14)}$=0.338, p=0.57), indicating that animals in both groups performed equally well when the food reward was not available in the pot compared to when it was. Thus, rats did not appear to use the scent of the food reward to solve the task. The significant main effect of group ($F_{(1,14)}$=6.390, p=0.02), indicates that even those rats in the LMNx group who reached criterion in the four-compartment stage of the task had impaired performance compared to rats in the Sham group. Thus, the animals in the LMNx group that completed all stages of training were less able than the sham animals to determine which compartment they were in at any given time and to recall the association between the compartment and the rewarded odor.

T-maze delayed spatial alternation

A delayed spatial alternation was used as an additional test of the directional sense. As can be seen from Figure 6, animals with lesions of the LMN were impaired consistently relative to sham lesioned animals. This difference was confirmed by a significant main effect of group ($F_{(1,18)}$=28.0, p<0.0005). Performance improved across training days for both groups, and there was a significant main effect of training day ($F_{(8,144)}$=2.53, p=0.013). However, the difference between groups did not interact with training day ($F_{(8,144)}$=0.69, p=0.70).

Progression in the odor-location conditional discrimination task was related to the extent of LMN lesion

As there was variability in the extent of the lesions in the LMNx group (Figure 2B), we investigated whether there was a relationship between the extent of the lesion and performance on the odor-location

conditional discrimination task (Figure 7). First, we compared the extent of tissue loss in the LMN and MMN in animals that had completed the task (i.e. reached criterion on the four-compartment stage) to those that had not (Figure 7A). A two-way mixed ANOVA revealed a significant interaction between brain area and completion status ($F_{(1,10)}$=32.274, p<0.0005), and a significant main effect of area ($F_{(1,10)}$=150.442, p<0.0005) but not completion status ($F_{(1,10)}$=1.910, p=0.197). Simple main effects were tested with one-way ANOVAs, and these revealed that animals who did not complete all stages of the task had significantly more tissue loss in the LMN than those that did complete this stage of the task ($F_{(1,10)}$=12.77, p=0.005). Conversely, there was no significant difference in the degree of MMN tissue loss between completers and non-completers ($F_{(1,10)}$=2.30, p=0.160).

Animals were given a completion score which related to the stage of the task they reached. Animals that completed the two-compartment stage only were given a completion score of 1, animals which completed both the two- and three-compartment stages were given a completion score of 2, and animals which completed all stages of the task were given a completion score of 3. Hence, we could investigate the degree to which lesion extent could be used to predict animals' progression through the task (Figure 7B). Linear regression analysis indicated that percentage tissue loss predicted completion score (R^2=0.663, $F_{(1,10)}$=19.671, p=0.001, Figure 7B). These analyses suggest that rats with greater LMN damage tended to progress less far in the task, whereas the degree of MMN damage was not associated with task progression (R^2=0.11, $F_{(1,10)}$=1.231, p=0.29).

Finally, we examined the relationship between LMN lesion extent and performance at each of the three training stages. LMN tissue loss did not predict the number of days required to reach criterion performance in the two-compartment stage in either the LMN (R^2=0.005, $F_{(1,10)}$=0.047, p=0.834, Figure 7C) or the MMN (R^2=0.001, $F_{(1,10)}$=0.013, p=0.911, data not shown). It did, however predict days taken to reach criterion in the three-compartment stage of the task (R^2=0.734, $F_{(1,10)}$=27.557, p<0.0005, Figure 7C). Again, no such relationship was found for MMN tissue loss (R^2=0.055, $F_{(1,10)}$=0.236, p=0.461, data not shown). In

the four-compartment stage of the task, there was a trend for animals with larger LMN lesions to require more days to reach criterion, but this did not reach the level of statistical significance (R^2=0.305, $F_{(1,10)}$=4.393, p=0.063, Figure 7C). Again, no correlation was observed between MMN lesions size and days to criterion was observed in this stage of training (R^2=0.051, $F_{(1,10)}$=0.538, p=0.480, data not shown). Together, these data suggest a greater degree of LMN damage was predictive of the time to reach criterion on the three-compartment, but not the two- or four-compartment, stage of the task, while degree of MMN damage does not predict days taken to reach criterion at any stage of the task. It should be noted, however, that only a subset of animals with LMN damage reached the four-compartment stage of the task.

Performance on the delayed spatial alternation task was related to the extent of LMN lesion

Data from the T-maze delayed spatial alternation task was examined to see if there was a similar relationship between tissue loss and task performance. Similar to the odor-location task, percentage tissue loss predicted mean percent correct choices over the final three days of testing on the T-maze (R^2=0.393, $F_{(1,10)}$=5.815, p=0.039). There was no significant correlation between percentage tissue loss in the MMN and percentage correct choices on the final three days of the T-maze task (R^2=0.014, $F_{(1,10)}$=0.125, p=0.732). These data imply that the extent of LMN damage, but not the extent of MMN damage, was predictive of the asymptotic T-maze delayed alternation performance achieved by the lesioned rats.

Discussion

This study tested the hypothesis that information provided by the head direction cell system is required for rats to discriminate between environments that differ only in their directional orientation. We found that rats with lesions of the LMN - a hub in the head direction cell system - were significantly impaired in the ability to acquire an odor-location task that required discrimination between identical compartments based on their orientation. Specifically, rats with LMN lesions took significantly more training sessions to discriminate between two compartments than control rats, and a significant proportion of lesioned rats failed to learn to discriminate between three and four compartments (whereas all control rats could do so). Rats with more extensive LMN

lesions showed greater impairments - they required more trials to reach criterion at the three and four compartment stages, and they were less likely to achieve criterion at these stages than rats with smaller LMN lesions. Animals with LMN lesions were also impaired in a delayed spatial non-matching to sample task on a T-Maze, and the magnitude of this impairment was also associated with LMN lesion extent in this task. Taken together, these findings suggest that the LMN supports the ability of rats to make discriminations based on directional information. We consider these findings and their implications in more detail in the following sections.

Lesions of the LMN impaired performance on a task requiring directional discriminations

Previous work has shown that place cells in the CA1 of the hippocampus (HPC) show place field repetition across visually identical maze compartments when these are oriented in the same direction (Grieves et al., 2016; Spiers et al., 2015). However, when compartments are arranged in a different direction to one another, as in the present study, there is no place field repetition (Grieves et al., 2016). This pattern of results suggest that the place cell system is driven by local inputs, such as the walls of a maze compartment, and a directional input. When the directional input is the same - as is the case when all maze compartments face the same direction - then both place cells and the animal's behavior reveal an inability to discriminate locations. A likely source of this directional input is the head direction cell system. Consistent with this, lesions of the lateral mammillary nuclei, a key node in this circuit, produce place field repetition in compartments that face different directions (Harland et al., 2017). The current results suggest that such lesions also impair the animals' ability to discriminate local environments based on a sense of direction at a behavioral level. Indeed, rats with LMN lesions in the current study showed remarkably similar performance (in terms of impaired task acquisition) to intact rats attempting to learn the odor-location conditional discrimination when all compartments face the same direction (Grieves et al., 2016). The impairment observed in the current study is unlikely to be due to an inability to

discriminate odors, or to associate odors with rewards, as the LMNx group were not impaired in the odor-shaping task compared to the Sham group.

The present findings indicate that lesions of the LMN impair acquisition of a conditional discrimination based on direction. Larger lesions were associated with increased difficulty in acquiring multiple discriminations, indicating that the odor-location task is sensitive to disruption of the head direction cell circuit. LMN lesions have previously been found to produce limited impairments on directional tasks in the T-maze and water maze (Aggleton et al., 1995; Harland et al., 2015; Harland et al., 2017; Vann, 2005, 2011). Even in a radial-arm maze task, which is a task with a similar arrangement to the one in our study, LMN lesions produced no impairment (Vann, 2018). However, in that study, extra-maze visual cues were available, so the animals could have used visual landmarks to solve the task in the absence of an internal directional sense. Studies in which lesions were performed in other nodes in the head direction network, such as the dorsal tegmental nucleus (Dwyer et al., 2013; Frohardt et al., 2006), anterior dorsal thalamus (Aggleton et al., 1996; Beracochea et al., 1989, Harvey et al., 2017), postsubiculum (Bett et al., 2012; Taube et al., 1992) and retrosplenial cortex (Pothuizen et al., 2008) have also yielded mixed results (for review see Dudchenko et al., 2019). Given the large number of brain areas containing head direction information, it is possible that there is a degree of redundancy in the HD system, such that some function can still be maintained even following lesions of a particular area. In addition, it is likely that behavioral tasks can be solved with different strategies, and only some of these strategies may depend on the integrity of the head direction circuit.

An outstanding question is why animals in which the head direction cell system has presumably been disrupted (via the LMN lesions) are still able to solve the first stage of the odor-location conditional discrimination task. The finding from this two-compartment stage is in agreement with our previous demonstration that non-lesioned animals learning the odor-location task in identical compartments that face the same direction were likewise able to acquire the two-compartment discrimination (Grieves et al.,

2016). This suggests that a simple discrimination (recognition that the current compartment is not the same as the one that the rat has just left) may be possible without directional information. A small proportion of animals in the Grieves et al. (2016) study were also able to acquire the four-compartment odor-location discrimination even when these faced the same direction.

One might predict also that the discrimination of compartments varies as a function of their angle to one another. Thus, the three-compartment stage may be more difficult than the two-compartment stage because, in addition to there being more compartments, the angle between them is decreased (from 120^{o} to 60^{o}, respectively). Likewise, it is certainly possible that in the current study animals with an impaired sense of direction from LMN lesions might have had difficulty with even the two-compartment stage if, for example, the angle between the compartments was reduced.

Although our results agree with these previous findings, the question remains unresolved as to how, in a situation where place field repetition is observed (or, in the case of this study, presumed to be occurring), can a subset of animals still discriminate between compartments. One possibility, as noted by Grieves et al., is that place fields may behave differently in the longer time frames associated with acquisition of the task (those animals in the LMNx group that did complete the task took between 31 and 60 days to do so) compared to the shorter time frames used in electrophysiological recording experiments. Indirect evidence for this idea comes from a study finding that over time, grid cells in the medial entorhinal cortex went from encoding local, repeated representations of two identical compartments to a single, global representation (Carpenter et al., 2015). It is therefore possible that on time scales comparable to those used in animals learning the task, place cells may be able to establish a more global firing pattern, even in the absence of directional information discriminating between compartments. Alternatively, it may be that the demands of the task encourage a subset of the rats to discriminate compartments, with repeated experience, based on subtle, non-directional cues.

Performance in the odor-location conditional discrimination task was sensitive to LMN lesion extent

We observed that the degree of tissue loss in the LMN significantly predicted how much of the task the rats were able to complete. We also observed that although most rats in the LMNx group had some degree of MMN tissue loss, there was no relationship between degree of MMN damage and performance on the task. Thus, the integrity of the LMN appears to be related to the capacity of rats to discriminate environments based on their angular orientation. We note, however, that despite this strong correlation there was one animal with a large LMN lesion who was able to complete the entire task. Thus, we can not preclude the possibility that with extensive training (which, for most animals would be beyond what they experienced in the current study), some strategy for solving the four-way discrimination that relies on subtle, non-directional cues could be acquired.

One caveat to the current findings is that the LMN does not contain HD cells exclusively (Yoder et al., 2015). Indeed, in recording studies only around 23-56% (Blair et al, 1998; Stackman and Taube, 1998; Yoder et al., 2015) of LMN single units encoded head direction, with the remainder thought to encode properties such as head pitch and angular head velocity. Thus, it is possible that the observed impairment on the odor-location task was not the result of removal of the directional signal alone. To address this, it would be of considerable interest to selectively inhibit the LMN neurons which project to the anterior dorsal thalamus, which contains a high proportion of head direction cells (Clark et al, 2009; Taube, 1995).

Lesions of the LMN impaired performance on a T-maze task

Given the relative novelty of the four-way odor-location conditional discrimination task, we were interested in testing whether our LMN lesions would replicate previous results seen by other groups in a more widely-used directional task. Hence, we tested animals on a non-matching to sample task on a T-maze. Previous work has shown the LMN lesions do not impair delayed non-matching-to-sample on a single T-maze (Vann, 2005, 2011), but alternation across two T-mazes is sensitive to LMN lesions (Vann,

2011). Surprisingly, we found a consistent impairment in alternation on a single T-maze with LMN lesions. As previous work has shown that a mild impairment in T-maze alternation is observed following lesions of the entire mammillary body complex, which contains the LMN and MMN (Aggleton et al., 1995), we can not preclude the possibility that incidental damage to the MMN contributed to this effect. However, as performance on the delayed alternation task was sensitive to the extent of LMN tissue loss, but not to the extent of MMN tissue loss, there is no clear evidence for this interpretation. An alternative possibility is that, as rats can engage in a variety of strategies in solving the T-maze, the configuration of the maze or the environment in the current study may have encouraged the use of directional information, and thus made it sensitive to LMN disruption.

Summary

The present study is a logical extension of previous work which showed that visually identical local environments which face different directions are easier to tell apart, both behaviorally and at the level of the brain's representation of location, than those facing the same direction. The current study addressed whether a key node in the head direction cell system, the LMN, is essential for the behavioral discrimination of such environments, and demonstrates that this is the case. Animals with LMN lesions are impaired in their acquisition of a four-way, odor-location conditional discrimination compared to animals with sham lesions. This finding complements a previous demonstration that LMN lesions similarly disrupt discrimination of local enviroments by place cells recorded in the hippocampus (Harland et al., 2017). Together, these findings suggest that a key function of the head direction cell system is to provide a directional reference that allows disambiguation of local environments.

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