

BRIEF REPORT

Spatial Learning in an Enclosed Eight-Arm Radial Maze in Rats with Sodium Arsanilate-Induced Labyrinthectomies

KLAUS-PETER OSSENKOPP*[†] AND ERIC L. HARGREAVES^{†1}**Neuroscience Program and [†]Department of Psychology, University of Western Ontario, London, Ontario, Canada N6A 5C2*

Bilateral vestibular dysfunction was induced in Long-Evans male rats ($n = 7$) by intratympanic injections of sodium arsanilate (30 mg/side). Control rats ($n = 6$) received isotonic saline. Animals were tested for labyrinthine integrity by measuring air-righting and contact-righting reflexes. Rats were reduced to 85% of free-feeding body weight and tested in an enclosed 8-arm radial maze (1 trial/day over 10 days). Labyrinthectomized animals made significantly more errors ($p < .001$) and, unlike the controls, showed no significant improvement on this measure over acquisition training. These rats also made significantly more ($p = 0.018$) sequential same arm reentries and fewer sequential adjacent arm entries ($p < .01$). These findings demonstrate that information obtained from the vestibular system is very important in spatial learning in the rat. © 1993 Academic Press, Inc.

The hypothesis that information obtained from the vestibular system is important in spatial orientation and learning has had a long history (see review in Potegal, 1982). However, until quite recently, little experimental evidence has been available to support this hypothesis. In many maze studies with rats the authors have argued for an important role for the vestibular sense in task acquisition, but most of these arguments have been based on indirect evidence (e.g., Zoladek & Roberts, 1978). More direct evidence has been supplied by maze studies which have used animals with vestibular dysfunction.

Beritoff (1965) reported that cats and dogs lacking

¹ Address reprint requests to Dr. K.-P. Ossenkopp, Department of Psychology, University of Western Ontario, London, Ontario, Canada N6A 5C2. This research was supported by an operating grant from the Natural Sciences and Engineering Research Council of Canada to the first author. Presented at the 19th Annual Meeting of the Society for Neuroscience, Phoenix, Arizona, 1989.

visual cues (blindfolded) and passively transported away from some designated starting point learned to return to this start point to obtain food reinforcement. More importantly, labyrinthectomy was shown to seriously impair performance in this passive transport and return task. A similar approach was developed, using rats as subjects, by Miller, Potegal, and Abraham (1983). Rats were trained to return to a water spout following passive transport away from this stimulus. Rats blinded by an enucleation procedure were successful in relearning the task, whereas rats with vestibular nucleus damage exhibited severe and lasting deficits.

Other studies have used various types of complex mazes to examine the role of vestibular cues in spatial learning. One study (Matthews, Ryu, & Bockanek, 1989) used a six-arm radial maze with a rotating central turntable. Rats were trained to locate a water reward which was located in a constant position relative to the starting position. No relevant visual, auditory, or olfactory cues were available in the apparatus. The animals were placed in the start position and then randomly rotated from 0 to $\pm 360^\circ$, with increments of 60° . Normal rats improved their performance of obtaining the reward over the training period. Rats with bilateral surgical labyrinthectomies displayed a profound impairment in their performance over trials. Thus, these results indicated that rats could discriminate among rotational stimuli of varying magnitude and direction and that these vestibular cues were used for navigational purposes and spatial learning. Schaeppi, Krinke, FitzGerald, and Classen (1991) used an unbaited six-arm radial tunnel maze to examine spatial learning in rats with streptomycin-induced vestibular dysfunction. Rats with vestibular dysfunction were significantly impaired in the order of

arm entries, relative to the control animals, providing additional evidence for the importance of vestibular cues in that task.

Sodium arsenite (Miller, 1985) has been shown to be effective in chemically labyrinthectomizing rats (Horn, DeWitt, & Nielson, 1981; Hunt, Miller, Nielson, & Horn, 1987; Ossenkopp, Prkacin, & Hargreaves, 1990; Porter, Pellis, & Meyer, 1990), guinea pigs (Anniko & Wersall, 1976), and meadow voles (Ossenkopp, Eckel, Hargreaves, & Kavaliers, 1992). Intratympanic injections of sodium arsenite have been shown to result in vestibular nerve degeneration in the brainstem (Anniko & Wersall, 1976) with concomitant loss of labyrinthine righting and reduced postural support with exaggerated head dorsiflexion (Horn et al., 1981; Hunt et al., 1987; Ossenkopp et al., 1990, 1992).

The present study examined spatial learning in the eight-arm radial maze in rats with chemical labyrinthectomies and in control animals. Zoladek and Roberts (1978) examined the sensory basis of spatial memory in this apparatus and concluded that visual cues and some types of internal cues (i.e., kinesthetic and/or vestibular) were important in this task. However, no direct test was made of the role of vestibular cues. The results reported in the present study provide evidence for the importance of vestibular information in this task.

Subjects. Thirteen adult male hooded rats (Long-Evans strain, Charles River, Quebec), weighing between 450 and 650 g, were individually housed in stainless steel wire mesh cages and kept in a colony room at $22 \pm 1^\circ\text{C}$ on a 12-h light:12-h dark cycle with lights on from 0700 to 1900 h. The animals were maintained at 85% of their free-feeding weight unless noted otherwise.

Behavioral apparatus. The eight-arm wooden radial maze consisted of a circular central platform, 30 cm in diameter, and eight identical arms. Each arm was enclosed and had dimensions of 80 cm (length) by 10 cm (width) by 16.5 cm (height). The arms were covered with Plexiglas lids attached by hinges and extended outward from the central platform (which was not covered) with adjacent arms being separated by 45° angles. Located at the end of each arm was a small 5-cm-diameter circular food dish attached to the floor. Apart from the Plexiglas lids the maze was painted a flat gray. A videocamera (Sony) was suspended above the maze and connected to a videomonitor and videorecorder for the purpose of viewing and videotaping of trials. The maze was located on the floor of the test room which contained a variety of distinctive visual cues.

Chemical labyrinthectomy. Sodium arsenite (atoxyl) was injected intratympanically following the procedure of Horn et al. (1981). Seven rats (Group VNX), under sodium pentobarbital anesthesia (Somnotol, 65 mg/kg, ip), received bilateral intratympanic 0.10 ml of sodium arsenite solution (300 mg/ml in sterile isotonic saline). Following each injection the ear canal was tightly packed with gelfoam. An additional six anesthetized rats received bilateral intratympanic injections of 0.10 ml isotonic saline (Group SHA).

Testing procedures. Following a 1-week recovery period the rats were tested for integrity of vestibular function. The first test examined the ability of the rats to right themselves in the air (air-righting reflex) when held supine and dropped from a height of approximately 45 cm onto a soft surface. A normal rat lands on its feet and a rat with labyrinth dysfunction will tend to land on its back or side. A second test (contact righting) examined the behavior of rats when placed supine on a horizontal surface and another horizontal surface (a sheet of Plexiglas) was lightly placed in contact with the soles of the supine animal's feet. Normal rats right themselves in this test, whereas rats with vestibular dysfunction will lie supine, with their backs in contact with the lower surface and their feet in contact with the ventral surface (Plexiglas sheet), and will not right themselves. Finally, all rats were tested for induction of rotation-induced nystagmus following several minutes of body rotation about a vertical axis at 70 rpm (see apparatus in Ossenkopp, MacRae, Bettin, & Kavaliers, 1988).

Ten days prior to acquisition training in the radial maze the animals were adjusted to 85% of their free-feeding weight and were then maintained at this level for the duration of the experiment. Each animal was tested in the radial maze apparatus with a single trial each day over a 10-day period. Each trial was begun by placing the animal in the fully baited maze at the end of a specific "home" arm. The rat was then allowed to run freely throughout the maze. On the first day of training each animal was left in the maze for a period of 15 min, independent of the number of arms entered. On subsequent days the animal was removed from the apparatus once all of the arms had been entered at least once and returned to its home cage. An arm "entry" was defined as a rat proceeding to the end of an arm. Videotape records of all trials were made and these were scored at a later time for the following variables: (a) number of arms entered until all eight arms had been entered at least once; (b)

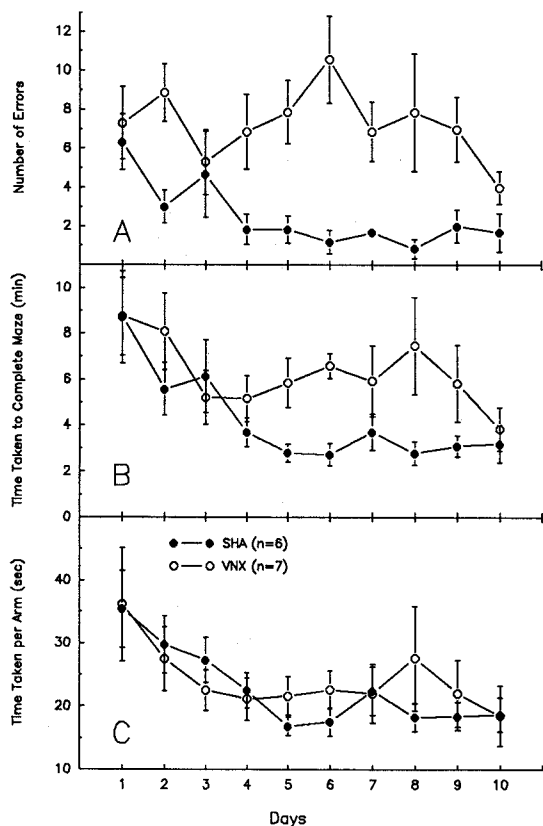


FIG. 1. Radial maze performance of control (SHA) and labyrinthectomized (VNX) rats as a function of days (trials) of task acquisition. (A) Group mean number of errors made. (B) Group mean time (minutes) taken to complete the maze task. (C) Group mean time (seconds) taken per arm visited. Error bars are SEM.

time (latency) taken until all eight arms had been entered at least once; (c) average time per arm entry (total time/number of arm entries); (d) number of sequential same-arm entries; (e) number of sequential adjacent-arm entries.

The data were analyzed with a mixed design repeated measures analysis of variance (SOLO, version 2.0, BMDP) and post hoc comparisons using Duncan's procedure. An alpha level of 0.05 was used in interpretation of statistical significance.

All of the rats treated with sodium arsenite exhibited clear loss of the air-righting reflex and failed to show the normal righting response in the contact-righting test. When challenged with vestibular stimulation by rotating the animals about a vertical axis, all of the normal rats (Group SHA) displayed rotation-induced nystagmus, whereas none of the atoxyl-treated rats (Group VNX) did.

The group mean errors (total number of arms entered minus eight) made in the maze task over acquisition days (trials) are depicted in Fig. 1A. Inspection of this figure suggests that Group VNX

made many more errors than did Group SHA, especially from Days 4 through 9. The results of the ANOVA test supported this impression by revealing a significant group (lesion) main effect ($F(1, 11) = 22.78, p < .001$). Although there was no significant main effect of trials ($F(9, 99) = 1.08, p > .05$) or a significant group by trials interaction ($F(9, 99) = 1.44, p = .10$), separate ANOVAs for the two groups revealed a significant trials main effect for the control group ($F(9, 45) = 2.84, p < .01$) but not the lesioned group ($F < 1$).

Figure 1B presents the group mean latency to complete the maze task as a function of days (trials). The only significant effect obtained in the ANOVA test was a trials main effect ($F(9, 99) = 3.43, p < .001$). When the group mean time spent in each arm was compared across acquisition trials (Fig. 1C), the statistical comparison indicated that the two groups did not differ across trials (group main effect; $F < 1$), but both groups took less time per arm entry over training trials (trials main effect; $F(9, 99) = 3.96, p < .001$).

The overall results for errors and latency to complete the maze suggest that Group VNX was impaired in the acquisition of the spatial task in terms of the number of errors. However, the lesioned group did eventually show improved performance in the maze as indicated by the significant improvement in time taken to complete the maze over training trials. It should also be noted that the two groups did not differ significantly in the amount of time spent in each arm.

The two other measures obtained from the videotapes, sequential same arm reentries and adjacent arm entries, revealed some interesting group differences in the way the spatial task was acquired. Figure 2A presents the mean group ratios of sequential adjacent arm entries/total arm entries. This ratio is a measure of the degree to which the rats perform the maze in an algorithmic fashion. Larger ratios represent more stereotyped performance in the maze. Statistical analysis with ANOVA revealed significant main effects of group ($F(1, 11) = 5.58, p = .038$) and trials ($F(9, 99) = 2.76, p = .006$), as well as a significant interaction for these variables ($F(9, 99) = 5.61, p < .001$). Inspection of Fig. 2A shows that Group SHA had increasing ratio values over trials, whereas Group VNX did not. Figure 2B depicts the mean frequency of same arm reentries for each group. Group SHA exhibited some same arm reentries on the first two trials but no reentries for the rest of the acquisition period. In contrast, Group VNX displayed sequential same arm reentries on all acquisition trials.

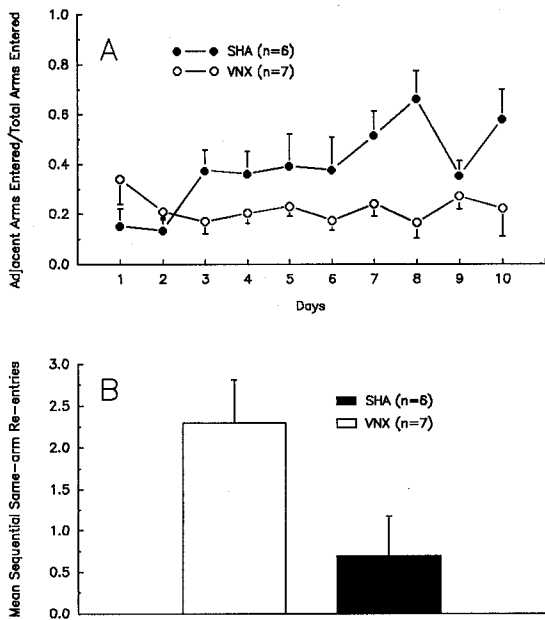


FIG. 2. (A) Group mean number of sequential adjacent arm entries as a ratio of total number of arms entered over days (trials) of task acquisition. (B) Group mean number of sequential same arm reentries during the entire acquisition period. Error bars are SEM.

Statistical comparison of the number of sequential same arm reentries for the two groups (Mann-Whitney test) indicated significantly more sequential same arm reentries by Group VNX ($p = .018$).

Results from the tests for labyrinthine integrity provided clear evidence for loss of vestibular function in the rats treated with sodium arsenite. The animals from Group VNX exhibited loss of the air-righting and contact-righting (Plexiglas sheet test with supine rats) reflexes, as well as absence of rotation-induced nystagmus. These findings are in agreement with previous studies which employed this chemical deafferentation procedure (e.g., Shoham, Chen, DeVietti, & Teitelbaum, 1989).

Loss of vestibular function produced some striking effects on behavior displayed during a spatial learning task in an eight-arm radial maze. The control (SHA) animals exhibited an optimal foraging strategy of collecting the food reward at the end of each arm with few reentries of arms already visited (cf. Olton, Collison, & Wertz, 1977; Zoladek & Roberts, 1978). In contrast, the labyrinthectomized (VNX) rats made many errors and showed no significant improvement on this measure over the task acquisition period. This striking impairment in task acquisition in the atoxyl-treated rats is consistent with previous studies of spatial learning in rats with vestibular dysfunction (Horn et al., 1981; Matthews

et al., 1989; Miller et al., 1983; Schaeppi et al., 1991).

The time taken to complete the maze task and the mean time per arm entry did not differ significantly between the two groups. Previous studies of sodium arsenite (Ossenkopp et al., 1990; Porter et al., 1990) and streptomycin-induced (Schaeppi et al., 1991) vestibular dysfunction in rats found increased levels of spontaneous locomotor activity, especially in the horizontal plane. Although such ototoxicity-induced hyperactivity may have influenced the acquisition of the spatial task, the time per arm entry measure does not provide any evidence for such a disruptive effect of hyperactivity, since the two groups were very similar on this measure. Furthermore, both groups exhibited equivalent decreases in time per arm entry over the acquisition period. Such significant improvements on this measure likely reflected acquisition of the instrumental task of obtaining food by running to the end of the arms, i.e., performance based on reference memory, but not necessarily acquisition of the spatial components of the optimal foraging task, based on working memory.

Two other measures, sequential same arm entries and adjacent arm entries, provided additional evidence for impaired spatial learning in the labyrinthectomized rats. Group VNX subjects made sequential same arm entries throughout the acquisition period, whereas the control rats exhibited this type of behavior only on the first two acquisition trials. In the radial maze recognition of arms already visited is considered to be a function of working memory (Olton, Becker, & Handelmann, 1979), one aspect of which requires sensory information related to the actual spatial location of the animal. In both the present study and a previous one (Schaeppi et al., 1991) with labyrinthectomized rats, working-memory-based performance was shown to be impaired. Loss of vestibular input thus seems to deprive animals of key sensory information needed for orientation in a complex maze. These findings are also consistent with previous research showing that specific, experimentally produced vestibular cues can be used by rats in locating a specific rewarding stimulus in a radial maze and that vestibular dysfunction impairs learning in such a task (Matthews et al., 1989).

The ratio of sequential adjacent arm entries to total arm entries is a measure of response stereotypy (algorithmic responding) previously observed in rats tested in a radial maze task (e.g., Dale & Innis, 1985; Roberts & Dale, 1981). Although efficient learning of the maze task does not require such

stereotypic adjacent arm entries (Olton & Samuelson, 1976), it is interesting to note that in the present study the control rats exhibited increasing scores on this measure over acquisition trials, whereas the VNX animals showed no significant improvement on this measure. The findings of the present study suggest that loss of vestibular information may have impaired the animal's ability to identify an arm that had just been entered or an adjacent arm, thus disrupting working memory, despite the availability of visual cues. Visual cues were available in the central platform area since this part of the maze was not covered and there were many distinctive visual cues in the test room. Given the importance attached to angular velocity signals from the vestibular system in landmark learning and sense of direction (McNaughton, Chen, & Markus, 1991), the deficits observed in the VNX rats are consistent with such an argument. However, it should also be noted that the labyrinthectomized rats probably had reduced visual input, due to the loss of the vestibulo-oculomotor reflex, and this may have contributed to the observed deficits as well. The present findings do not discriminate between impairment of egocentric spatial navigation, based on internal cues (e.g., vestibular), and impairment of allocentric spatial learning, based on use of external cues. Future studies need to examine the relative contributions of visual and vestibular cues to performance in a radial-maze task by selective removal of these sensory cues.

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