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Olfactory Bulbectomy Temporarily Impairs Morris Maze Performance: An ACTH(4-9) analog Accellerates Return of Function

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VAN RIJZINGEN, I. M. S., W. H. GISPEN AND B. M. SPRUIJT. *Olfactory bulbectomy temporarily impairs Morris maze acquisition: An ACTH(4-9) analog accellerates return of function.* PHYSIOL BEHAV **58**(1) 147–152, 1995.—Rats with a bilateral lesion of the olfactory bulb are permanently anosmic. However, this lesion also produces nonspecific behavioral effects that recover over time. In this study olfactory bulb-lesioned animals are given a spatial orientation task—the Morris maze—which supposedly relies on visual and not olfactory cues. In exp. 1 this assumption was verified by subjecting animals with peripherally induced anosmia to the Morris maze (olfactory neurons in the nasal mucosae were destroyed by flushing the nose with ZnSO4). Anosmia did not affect the acquisition rate of the animals. In exp. 2 anosmia was produced by a central lesion to the bulbus olfactorius. Two weeks after lesioning the Morris maze performance is severely impaired. Interestingly, chronic administration (10μg/48 h/rat, during these 14 days, SC) of the ACTH(4-9) analog ORG 2766 diminished the impairment in performance. In exp. 3 olfactory bulb-lesioned animals were allowed 6 wk to recover before Morris maze testing began, to investigate if spontaneous recovery of performance occurred. No difference was seen in the acquisiton performance of lesioned animals when compared to sham animals at this timepoint. The effect of the peptide is discussed in the context of an accelleration of the recovery of non-specific consequences of brain lesioning.

Bulbus olfactorius ORG 2766 Functional recovery Morris maze Rat Learning Lesion

INTRODUCTION

ADMINISTRATION of the ACTH(4-9) analog Org 2766 can influence recovery after damage to the central nervous system (13,18,26,30). In several studies the peptide effect has been ascribed to an accelleration of spontaneously occurring recovery processes, such as denervation supersensitivity or restoration of dopaminergic innervation (30). However, in a previous study we have demonstrated that a beneficial effect of the peptide could also be established in a model where spontaneous functional recovery does not occur (manuscript in preparation). The peptide was supposed to stimulate compensatory processes by inducing a state of enhanced nonselective attention. Since the effect of ORG 2766 occured irrespective of the occurrence of spontaneous recovery, nonspecific functional damage (lesion-induced behavioral deficiencies not related to the afflicted brain region) should also be susceptible to treatment. A lesion model suitable to test this hypothesis is the bilateral olfactory bulb lesion, which is known to produce temporary nonspecific behavioral effects. The olfactory bulb (OB) contains the primary processing unit for olfactory stimuli (5), but lesioning of this area also affects many nonolfactory mediated behaviors, such as mouse-killing (6,22), aggression (1,8), reactivity to stimuli (8) and learning (4,5,12,24,25). Bulbectomized animals (OB) show an accelleration of active avoidance learning, but a deficit in passive avoidance learning. Prelearned tasks are not affected by subsequent surgery. Interestingly, nonolfactory mediated behavioral deficits (such as impairment of passive avoidance learning and spontaneous alternation) vanish within 4/5 wk (8,4), whereas olfactory-related deficits such as odor-preference are permanent (15). This indicates that the direct functions of learning and memory are probably still intact, but the OB-lesion blocks them temporarily. The cause of this obstruction is not known, although in several studies on olfactory bulbectomy the induced impairments have been ascribed to a state of hyperemotionality (hyperreactivity to stimuli) or to a state of deficient arousal.

The present study combines the OB-lesion with the Morris maze, a test which supposedly relies on visual and not olfactory cues, to investigate if the OB lesion can induce nonspecific temporal disruptions of function due to central lesioning. In exp. 1 it was first verified if anosmia, produced without damaging the brain, could interfere with Morris maze performance. Anosmia was produced by nose flushing with ZnSO4, which destroys the olfactory neurons in the nasal mucosae.

In exp. 2 the effect of the OB-lesion on Morris maze performance was investigated, and the ACTH(4-9) analogue was ad-

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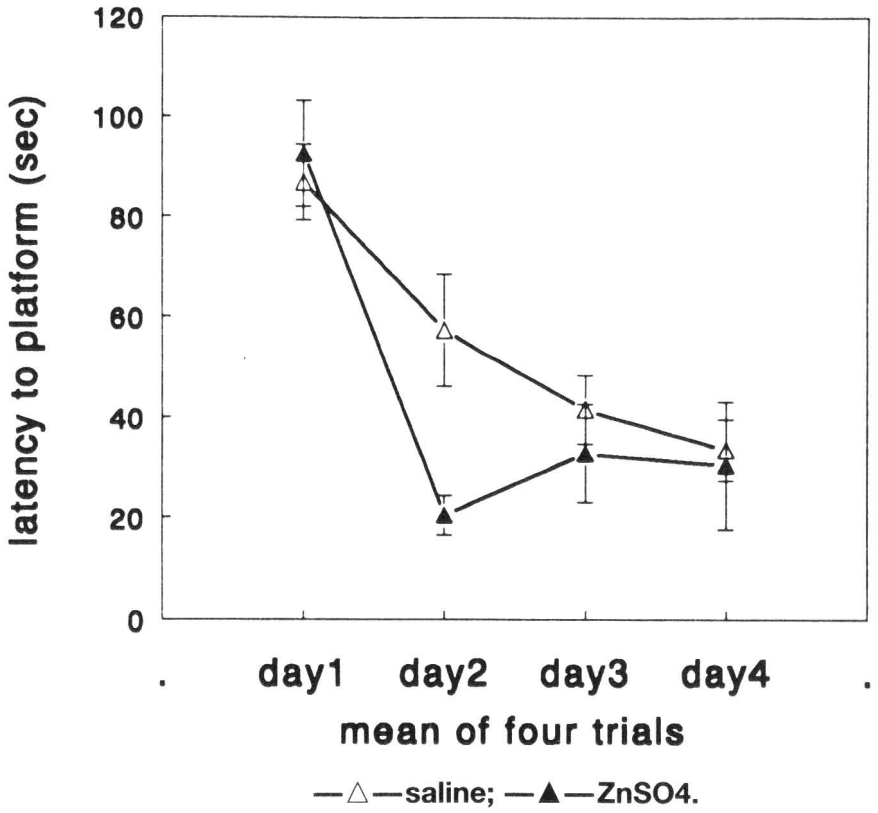

FIG. 1. Morris maze acquisition of peripherally induced anosmic animals. Mean latency to reach a submerged platform over four trials is plotted against the days of testing. Saline = saline flushed animals, ZnSO4 = ZnSO4 flushed animals-anosmic.

ministered to see if peptide administration could affect the performance of lesioned animals. Finally, in exp. 3 OB-lesioned animals were allowed 6 wk to recover (instead of 2 wk in exp. 2) before Morris maze testing to asses if spontaneous recovery of the OB-lesion induced effects could occur.

MATERIALS AND METHODS

Subjects

Male Wistar rats (obtained from R.M.I, Utrecht) were group-housed (3 rats per cage) in macrolon cages with sawdust bedding and ad lib rat chow and water, and kept on a reversed day-night cycle (lights off from 8.00 h to 20.00 h).

Lesioning. Olfactory Bulbectomy (OB). All animals were anaesthetized with an intramuscular injection of Hypnorm (Duphar,Weesp NL) which contains flunisone (10mg/ml) and pentanylcitrate (0.2 mg/ml) in combination with a SC injection with Diazepam (midazolam 5 mg/ml) and placed in a stereotactic unit. The skull was exposed. A slit was drilled perpendicular to the line Lambda-Bregma, 6.9 mm anterior from Bregma. A bilateral transection was performed by a sharp scalpel no 13. Sham animals were treated identically except for the actual incision. All animals received a SC injection with 0.5 ml saline to make up for blood loss. All animals were tested once for anosmia by the "apple retrieval test." For purposes of analysis a rat was considered anosmic if it failed to locate a buried piece of apple it it's home cage within 5 min. After behavioral testing all animals were sacrificed. The olfactory bulb and the rostral area of cortex were exposed. Lesions were visually verified. If transection was not complete, or the cortex was damaged the data of this animal was excluded from analysis.

ZnSO4-flushing. The rats received ether anaesthesia and the nose was flushed with saline or ZnSO4 (5% $ZnSO_4 \cdot 7\ H_2O$) solution until fluid emerged from the nose. The animals were allowed one day to recover before behavioral testing. All animals were tested daily for return of olfaction by the "apple retrieval test."

Morris maze. A Morris maze apparatus was used with a black pool (diameter 200 cm, depth 40 cm) filled with water (temperature 27°C ± 1°C). Low red light conditions resulted in a black water surface in which the black platform could not be detected. In quadrant 1 of 4 fixed quadrants a black escape platform was placed 1 cm below the surface. Cues were provided by two white boards with black signs placed around the rim of the pool. Observations were made using an automated system described below. All rats received 16 trials during acquisition training, 4 trials each day on four consecutive days with an intertrial interval of 10 min. A rat was entered facing the wall at random on one of four entrypoints, and allowed to swim for 120 s. The latency till the rat mounted the platform was recorded. If the rat failed to find the platform within 120 s it was guided there by hand (latency was set at 120 s) and allowed to remain on the platform for 30 s. On the fifth day the platform was removed. All rats entered at a fixed point and allowed 60 s to swim, during which their swimming pattern was recorded by the automated system. During analysis the time and travelled distance in two zones was

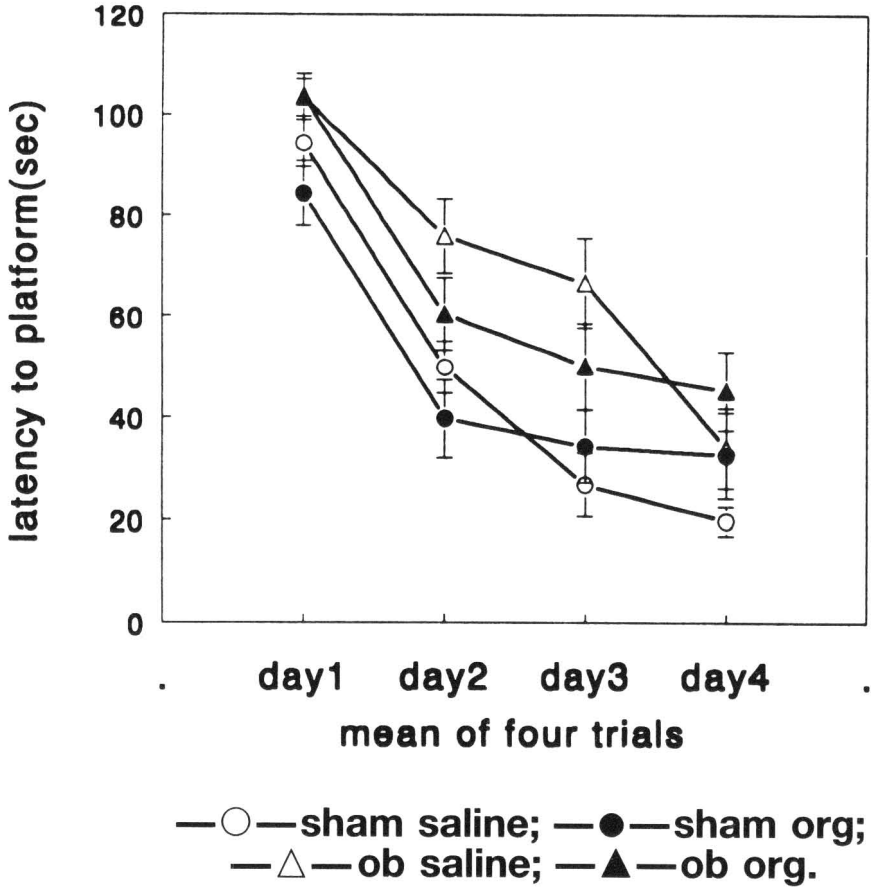

FIG. 2. Morris maze acquisition of OB lesioned animals tested 2 wk after the lesion (exp. 2). Half the animals received ORG 2766 treatment (10 microgram/animal/48 h, 14 days). Sham\saline = sham operated animals treated with saline. Sham\ORG = sham operated animals treated with ORG 2766. OB\saline = bilateral lesion of the olfactory bulb, treated with saline. OB\ORG = bilateral lesion of the olfactory bulb, treated with ORG 2766.

measured: one circle of 20 cm around the platform position (platform zone) and one zone of equal size on the opposite side of the pool (reference zone).

Automated system. The output of a video camera, mounted above the centre of the pool, was directly fed into a computerized image analysis system that records the rats position approximately twice per second. After defining zones in the recorded field, the time and travelled distance per zone and the latency to enter each zone can be calculated by the program. Hardware consisted of an IBM AT computer, combined with a PC vision frame grabber (Imaging technology Inc. USA) and a CDD video camera. The software was developed by Noldus Information Technology, Wageningen, The Netherlands (28).

EXPERIMENTAL PROCEDURES PER EXPERIMENT

Experiment 1: ZnSO4 Flushing

Twenty-one animals were randomly assigned to two groups. Ten animals received ZnSO4- flushing, the other eleven were flushed with saline. All animals were given one day to recover before Morris maze testing began. The data was analyzed statistically with a one-way Analysis of Variance with repeated measurements. No probe trial was conducted since six days after flushing all animals readily retrieved the apple in the anosmia test.

Experiment 2: OB-Lesion and ORG 2766

Fifty-six animals were randomly assigned to four groups. Sham operated animals and OB-lesioned animals received either SC injections with saline (0.5 ml/rat/48 h) or ORG 2766 (10 μg/0.5 ml saline /48 h) for fourteen days. The group sizes were: sham-saline n = 14, sham-org n = 15, OB-saline n = 14, OB-org n = 13. Fourteen days after the lesion behavioral testing in the Morris Maze started. Statistical analysis for the acquisition data was performed by a Two-way Analysis of Variance with repeated Measurements, and of the probe trial by a Two-way Analysis of Variance, both over the factors lesion and treatment.

Experiment 3: OB-Lesion and 6 Weeks

Twenty-one animals were randomly assigned to either a sham lesion or a OB-lesion. All animals were allowed 6 wk to recover before Morris maze testing began. The day was analyzed with a one-way Analysis of Variance with repeated measurements.

RESULTS

Histology

In the groups of rats receiving OB-lesions, OB tissue posterior to the site of the lesion (1–1.5 mm posterior to the cortex) had

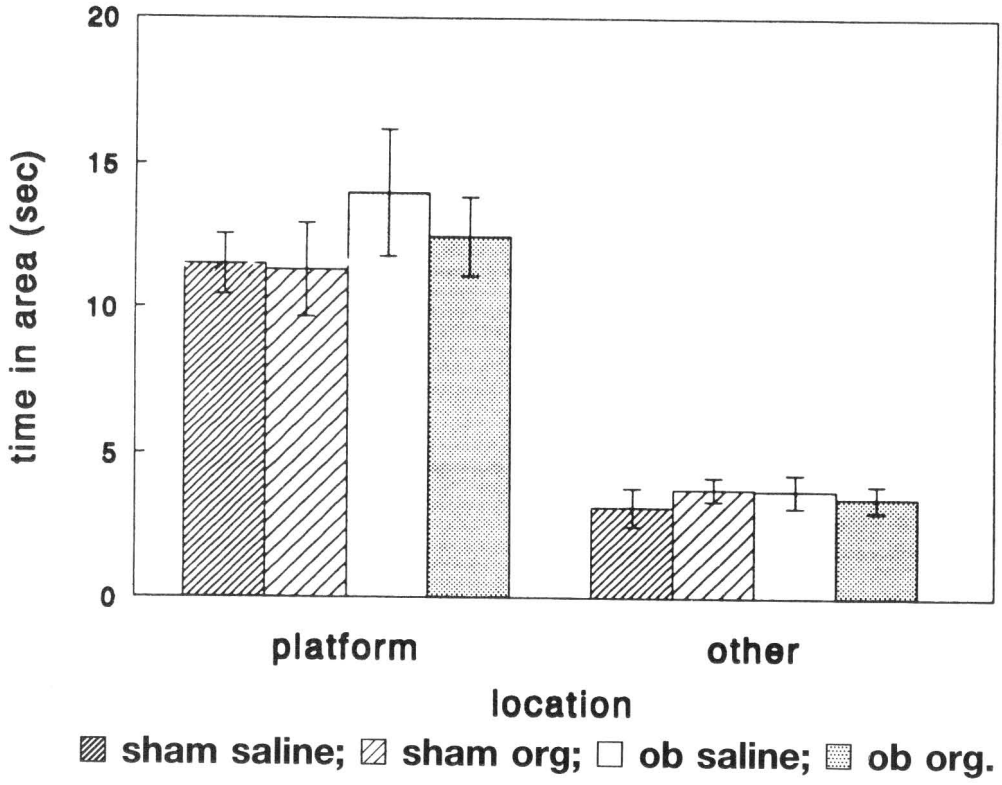

FIG. 3. Probe trial data of the groups from exp. 2. Half the animals were treated after the lesion with ORG 2766 (10 microgram/animal/24 h). The graph represents the time the animal spent in either the platform zone: a circle with a radius of 30 cm around the location of the platform, or in the other zone: a reference zone of equal size on the opposite side of the pool. Sham\saline = sham operated animals treated with saline. Sham\ORG = sham operated animals treated with ORG 2766. OB\saline = bilateral lesion of the olfactory bulb, treated with saline. OB\ORG = bilateral lesion of the olfactory bulb, treated with ORG 2766.

completely degenerated upon inspection, thus effectively removing the major portion of OB tissue.

Experiment 1

The Morris maze acquisition performance of anosmic (ZnSO4-flushed) animals did not differ significantly from saline flushed animals, (Fig. 1). Peripherally induced anosmia clearly did not impair the performance in the Morris maze task.

Experiment 2

Figure 2 shows the performance during the acquisition phase of the Morris maze. All sham operated animals learned the task in four days [repeated measurements $F(1, 150) = 170.320$ $p < .001$]. OB-lesioned animals were slower in finding the escape platform, [lesion $F(1, 150) = 17.228$ $p < .001$] although the latencies of OB-lesioned animals treated with ORG 2766 declined faster than those of saline treated counterparts [repeated measurements × lesion × treatment $F(3, 150) = 3.593$ $p < .015$]. In the probe trial (Fig. 3) all animals showed preference for the previous location of the platform over the reference zone.

Experiment 3

The Morris maze performance of OB-lesioned animals and sham controls 6 wk after surgery did not show a difference in escape latency during acquisition (Fig. 4), nor any difference during the probe trial. All animals spent more time in the previously reinforced area than in the reference area (graph not shown).

DISCUSSION

The present results illustrate that bilateral lesioning of the olfactory bulb produced a transient impairment in acquisition of the Morris maze (exp. 2), but that anosmia was probably not the cause of this impairment (exp. 1). Evidently, the disruption of performance is a nonspecific consequence of the brain damage. Six weeks after the lesion spontaneous recovery of the Morris maze impairment has occurred (exp. 3). It is unlikely that the recovery of function arised from any regenerative process that results in resumed efficiency of the OB, since all OB tissue had degenerated in lesioned animals 2 wk after the lesion. Interestingly, administration of ORG 2766 2 wk subsequent to surgery accellerated the recovery from the nonspecific consequences of the OB lesion. The efficacy of the peptide could evidently not be ascribed to it's supposed neurotrophic function (11,30). To determine the nature of the peptide effect the cause of the transient impoverishment should be considered. Two alternative explanations have been forwarded, based on the lesion effects on sexual behavior and avoidance conditioning.

First, OB-lesioned animals show abnormal sexual behavior (9,14). Cain and Paxinos concluded that nonolfactory influences of the olfactory bulb contribute to sexual arousal necessary for normal sexual behavior (6). Extrinsic stressors can overcome this

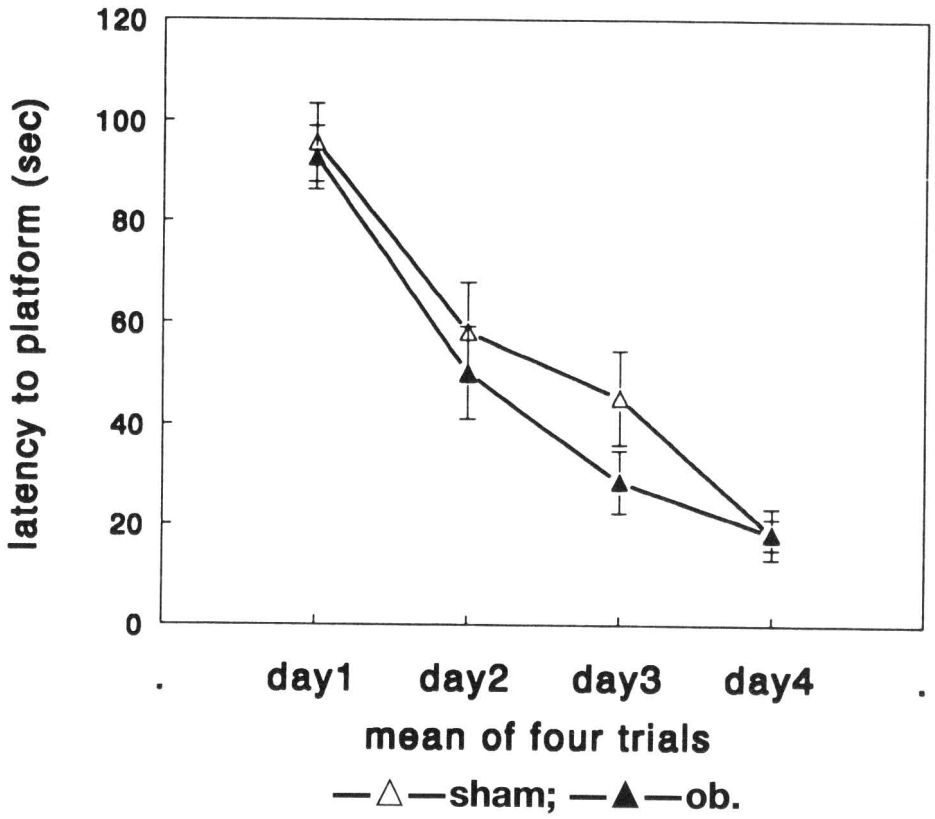

FIG. 4. Morris maze acquisition of OB lesioned animals tested 6 wk after the lesion. The graph represents the mean latency to reach a submerged platform over four trials plotted against the days of testing. Sham = sham operated animals, OB = animals with a bilateral lesion of the olfactory bulb.

arousal deficiency, tail pinch, and flank shock invoke normal sexual behavior (2,20). However, it is unlikely that the impaired performance in the Morris maze is caused by an arousal deficiency, since water immersion can be considered at least as severe a stressor as flank shock or tail pinch, and should overcome the problem. Alternatively, Gomita et.al have suggested that the impairment of discriminiation avoidance conditioning after OB lesioning ensued from a state of hyperemotionality induced by OB-lesioning (12). Such a general state could also account for the impairment in Morris maze performance found in the present study. Interestingly, the ACTH (4-9) analog can diminish a septal-lesion-induced state of hyperemotionality (15), which could explain the present peptide efficacy. However, the influence of the peptide on lesion-induced behavioral changes and on human cognition both have been explained before by an enhancement of attention to external stimuli (3,19). Undoubdetly a state of hyperemotionality is not beneficial for attention to stimuli. Through an enhancement of attention ORG 2766 could have compensated for the disorienting effects of an exagerrated emotionality/reactivity.

A neuronal substrate for the peptide has not yet been found, although it has been shown that ACTH can bind to the NMDA-receptor (29), and ORG 2766 can counteract the behavioral effects of overactivation of the NMDA-receptor system induced by icv-administration of NMDA (27). NMDA-receptors are involved in neurotoxicity after mechanical damage as well as after ischemia, status epilepticus and hypoglycemia (7). Axotomy induces a massive release or leakage of neurotransmitters, including Excitatory Amino Acids (EAA's), which results in cell depolarization in neuronal tissue. It is hypothesized that EAA's overactivate the NMDA receptors. Excessive excitation of brain structures can cause suppression of the normal function of these structures and subsequent desorientation and loss of control of the animal. The hippocampus is known to contain a relatively high concentration of NMDA-receptors (21) and is thought to be involved in spatial orientation (23). A suppression of the normal function of the hippocampus as a consequence of the OB-lesion may induce the impairment in spatial orientation. The peptide may excert its beneficial effect by a direct or indirect interference with the hippocampal overexcitation. This theory is in agreement with the effects of the peptide seen after septal lesions (16), NMDA-treatment (27) and ageing. The characteristics of the OB lesion induced impairments; transsynaptic effects in remoter parts of the brain, and the reversibility of these effects, are reminiscent of the originally clinical term diaschisis (neuronal sparing). While introduced by Von Monakow in 1914, this term was redefined by Finger and Stein (10) as "the temporary dispersion of function unrelated to the site of damage." LeVere (17) interpreted the recovery of aphagia after lateral hypothalamus lesions as 'recovery of function which does not reflect functional reorganizing of neuronal tissue, but rather the survival of neural tissue that mediated the behavioral patterns prior to the lesion'. In view of these definitions, the behavioral effects after OB lesioning could be considered an appropriate animal model for diaschisis.

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