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Effects of Chronic Resistive Airway Loading on Behavioral Changes in Rats

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Abstract

Complete vascular ring is a term describing a group of congenital aortic arch abnormalities that is often observed in infancy and leads to tracheoesophageal compression and airway obstruction. We investigated the effects of chronic resistive airway loading on anxiety and learning behavior in Wistar rats. The trachea were obstructed by a circumferential tracheal band to increase respiratory esophageal pressure. All of the rats were subjected to the elevated plus-maze test and the active avoidance test at 2 or 4 weeks after the obstruction surgery. One set of rats received surgical relief of the tracheal obstruction on week 2 or 3 after obstruction. At 4, but not 2, weeks after tracheal obstruction, the rats showed impairment of learning in the active avoidance test and this impairment was prevented by surgical relief of tracheal obstruction performed on week 2, but not week 3. In contrast, anxiety-like behavior in the elevated plus-maze test was not affected at either 2 or 4 weeks after tracheal obstruction. Body weight was not affected by tracheal obstruction and no differences were seen in arterial blood gases after 4 weeks of tracheal obstruction. These results suggest that chronic tracheal obstruction causes learning deficits and that surgical intervention is necessary and should be performed as early as possible to prevent long-term sequelae.

Key Words: complete vascular ring, tracheal obstruction, chronic resistive airway loading, anxiety, learning

Introduction

Several respiratory disorders, such as systemic inflammation in chronic obstructive pulmonary disease, obstructive sleep apnea syndrome, tracheomalacia, tracheal stenosis and upper airway resistance syndrome, can lead to chronic resistive airway loading (1). Complete vascular ring (CVR), a congenital anomaly of the aortic arch, encircles the esophagus and trachea and leads to tracheoesophageal compression and obstruction, which can cause life-threatening respiratory distress in early infancy (5, 21). Patients

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with congenital airway obstruction in the laryngeal or tracheal region may show their first symptoms at school age. The increased respiratory resistance caused by central airway obstruction, frequently misdiagnosed as asthma (7, 15, 27), increases the risk of respiratory disturbance (16, 22, 24, 26). Untreated CVR has been reported to result in cognitive and behavioral dysfunction (2, 11). An animal model of airway obstruction is needed to study the links between CVR and behavioral changes and for the development of early diagnostic and therapeutic strategies.

Both the elevated plus-maze (EPM) test and the active avoidance test are widely used in biopsychological studies. The time spent in the open arm during the EPM test is used to evaluate unconditioned avoidance behavior as a measure of anxiety-like behavior (4, 17, 28), while the active avoidance test is used to examine learning ability (3, 23). When animals face an avoidable stress, for example an electrical shock to the foot, they may learn to cope with it by performing an appropriate response leading to avoiding, or escaping from, the noxious stimulus. The aims of this study were to develop an animal model for tracheal obstruction, to investigate tracheal obstruction-induced long-term effects on anxiety and learning behavior in the EPM test and active avoidance test, respectively, and to examine the optimal timing for surgical intervention to relieve tracheal obstruction and avoid sequelae.

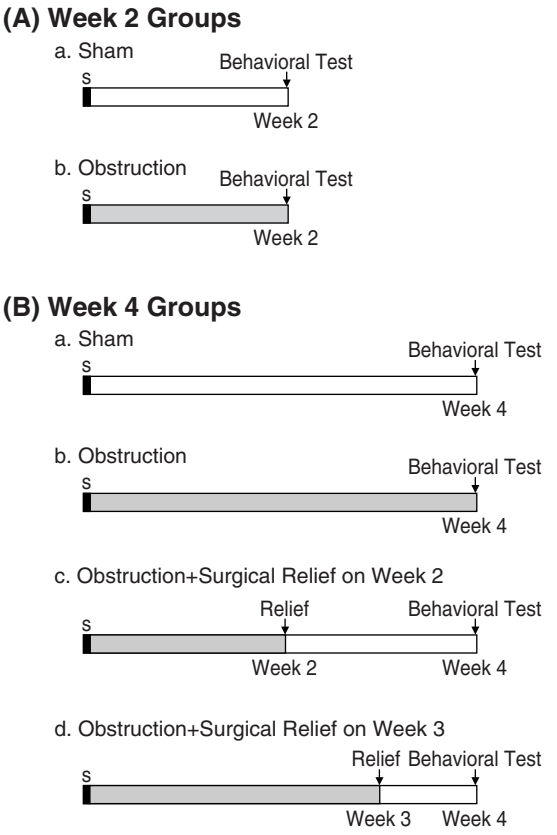

Fig. 1. Flowchart of the grouping and treatments. S is surgery. The gray bar shows tracheal obstruction and the white bar no tracheal obstruction.

Materials and Methods

Animals and Procedures

Male Wistar rats (12-week old, 388.6 ± 3.1 *g*; n = 104; National Laboratory Animal Breeding and Research Center, Taiwan, ROC) were housed in groups of five in acrylic cages (35 × 56 × 19 cm) in an animal room with controlled temperature (24 ± 1°C) and humidity (55 ± 5%) under a 12 h light-dark cycle (lights on at 07:00 a.m.) with food and water available *ad libitum*. Each animal was handled for 5 min/day on 3 consecutive days prior to the experiment. All experimental procedures were performed according to the NIH Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH publication No. 85-23, revised 1996) and were approved by the Animal Care Committee of the Chung Shan Medical University (IACUC Approval No.: 493).

In the tracheal obstruction group, increased tracheal resistance was imposed by circumferential tracheal binding, while the control animals underwent sham operation (see below). At 2 or 4 weeks after tracheal obstruction, the animals were observed for anxiety-like and learning behavior in the EPM test and active avoidance test, respectively. Forty-five rats from the week 4 group were assigned to receive surgical relief of tracheal obstruction at week 2 or 3 after tracheal obstruction. Theses two sub-groups were also observed for behavior at week 4. Body weight was measured once per week. The schedules for the surgery and behavioral tests are shown in Fig. 1.

Tracheal Obstruction Surgery

The method of tracheal obstruction surgery was adapted from that reported by Tarasiuk and Segev (31). Aseptic surgical procedures were employed for all rats. The rats were anesthetized with 2% isoflurane and placed on an operating table. Body temperature was maintained at 37 ± 0.5°C during surgery with a heating pad servo-controlled by a rectal probe. A saline-filled catheter was placed in the esophagus and connected to a pressure transducer to measure respiratory swings and esophageal pressure. The trachea was exposed by midline ventral cervical incision without damaging adjacent structures including the recurrent laryngeal nerve and the region adjacent to the carotid bodies. A 0.5-cm long circumferential plastic band was placed around the trachea and a suture looped around the band and trachea and tight-

ened, thus, constricting the trachea so as to increase inspiratory esophageal pressure swings by 2- to 3-fold. Immediately after surgery, the rats were injected with penicillin-G procaine (0.2 ml, 20,000 IU, IM) and housed individually in plastic cages (25 × 41 × 19 cm) for 7 days, then were re-grouped in their home cages (rats from the same home cage underwent the same treatment).

Behavioral Tests

At week 2 or 4 after surgery, the rats were subjected to the EPM test followed by the active avoidance test (20 trials). All behavioral tests were performed between 10:00 and 16:00. The animals were weighed in the animal room, placed individually in a clean cage (25 × 41 × 19 cm), and transported to a dim observation room (28 lux) for behavioral testing. The test equipment was cleaned using 20% ethanol and thoroughly dried before each trial.

EPM Test

Behavior in the EPM test was observed for 5 min, as described in our previous reports (17-19). The following measurements were made from videotapes: (a) arm time, the time spent in the open or enclosed arms; (b) arm entries, the number of entries into the open or enclosed arm; and (c) risk assessment, the rat showed head dipping, but its body was still in the enclosed arm during this behavior. Entry into any compartment was defined as all four paws being placed in the compartment.

Active Avoidance Test

Thirty minutes after the EPM test, the active avoidance test was performed in a shuttle box (AccuSan, USA), consisting of two equal compartments (25 × 25 × 45 cm) with a grid floor made of stainless steel bars separated by a wall with a central door (6 × 6 cm). The rats were randomly placed in one of the compartments with the central door open, allowing them to explore the compartments for 1 min, then underwent 20 shuttle trials, each lasting 1 min, performed as in our previous reports (19, 23). The conditioned stimuli (CS; 75 db tone plus 250 lux light for 3 sec) and unconditioned stimuli (UCS; 0.5 mA of scrambled foot shock for 10 sec) were delivered and controlled by a computer. During the test, the rats needed to cross from one compartment of the shuttle box to the other to avoid or escape the shock. Each trial began with a 3 sec CS, which was followed by the UCS of foot shock. If the rat passed through the door during the CS, the CS was terminated, no shock was delivered, and an avoidance response was recorded. If the rat passed through the door during shock delivery, the shock was terminated and an escape response was recorded. If the rat did not go through the door, the shock was terminated after 10 sec and a failure response was recorded. After an interval of 47-57 sec controlled by the computer, the next trial was initiated. The latency to avoiding or escaping the shock and the number of avoidance responses, escape responses and failure responses was recorded.

Data Analysis

Statistical testing was performed to compare within or between groups using *t*-tests for paired or unpaired data. Comparisons between 3 or more groups were performed using one-way analysis of variance (ANOVA), followed by the least-significant difference *post hoc* test. Analysis of the effects of tracheal obstruction on changes in body weight and escape latency in the active avoidance test was carried out by ANOVA repeated measures. The level of significance was defined as $P < 0.05$. All results are expressed as the means ± SEM.

Results

Immediately after tracheal obstruction, the respiratory rate decreased ($P < 0.001$) and the difference in esophageal pressure in respiratory swings increased significantly ($P < 0.001$) compared to the sham-operated group (Table 1). Audible wheezing was noticed in rats with chronic resistive airway loading, especially after movement activity, but no signs of gasping were observed. Four weeks after surgery, no differences in the concentration of arterial blood gases were observed between tracheal-obstructed rats and sham-operated rats (Table 2). Body weight was not affected by tracheal surgery (Fig. 2, A and B). The behavior of the rats in the EPM test, performed at week 2 or 4 after tracheal obstruction, was not different from that of the sham-operated rats irrespective of whether or not they received surgical relief on week 2 or 3 (Table 3).

Two weeks after tracheal obstruction, the escape latency in the active avoidance test showed a significant time effect [$F(19,323) = 4.41$, $P < 0.001$] and surgery effect [$F(1,17) = 134.09$, $P < 0.001$] (Fig. 3A). Similar phenomena were observed 4 weeks after obstruction with a time effect [$F(19,1520) = 11.24$, $P < 0.001$], surgery effect [$F(1,80) = 797.89$, $P < 0.001$] and time-by-surgery interaction [$F(57,1520) = 1.62$, $P = 0.003$] (Fig. 3B).

In the test performed 2 weeks after surgery, the mean escape latency in the active avoidance test was shortened from the first 10 trials to the second 10 trials in both sham-operated rats (7.1 ± 0.7 sec to 5.6 ±

Table 1. Effects of tracheal obstruction on respiratory rate and esophageal pressure

	Sham Operation (n = 8)	Tracheal Obstruction (n = 8)
Respiratory rate (breaths/min)		
before	65.01 ± 1.65	66.11 ± 1.49
after	64.85 ± 1.45	56.61 ± 1.38***
ΔPes (cm H_2O)[a]		
before	-8.45 ± 0.28	-8.54 ± 0.40
after	-8.48 ± 0.39	-16.54 ± 1.02***

The data are the values before and immediately after surgery. [a]difference in esophageal pressure in respiratory swings. ***Significantly different from the data before surgery ($P < 0.001$, paired t-test).

Table 2. Lack of effect of tracheal obstruction on arterial blood gases at 4 weeks after surgery

	Sham Operation (n = 4)	Tracheal Obstruction (n = 5)	*P* Value
pH	7.42 ± 0.01	7.35 ± 0.06	0.43
PO_2 (Torr)	107.00 ± 2.65	104.00 ± 6.57	0.75
PCO_2 (Torr)	42.00 ± 2.08	44.80 ± 3.85	0.62
HCO_3^- (meq/l)[a]	27.00 ± 0.96	26.88 ± 1.26	0.95

[a]HCO_3^-, calculated from arterial bicarbonate.

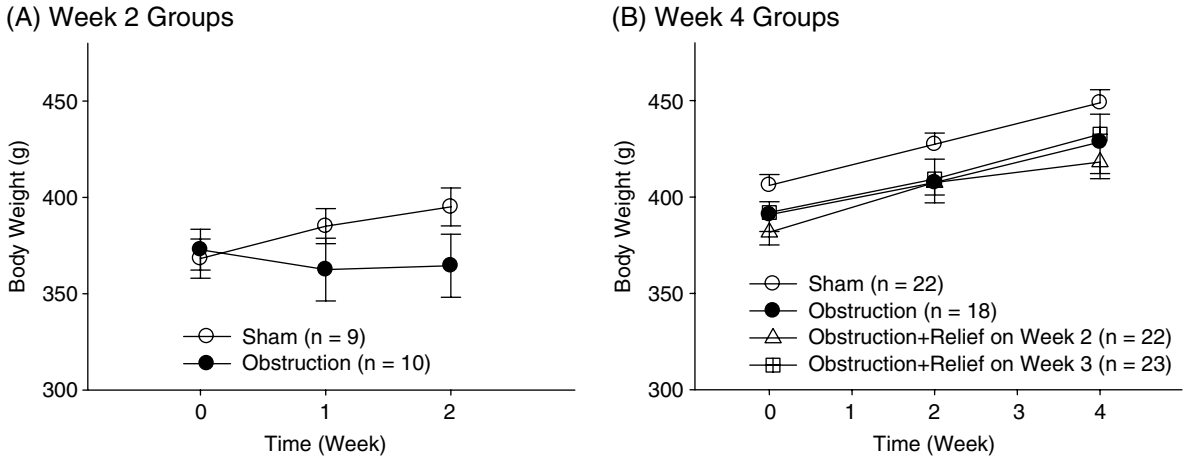

Fig. 2. Lack of effect of tracheal obstruction on body weight. Average body weights were measured 2 (A) or 4 weeks (B) after tracheal obstruction. The data are means ± SEM.

1.0 sec) (df = 8, t = 2.69, $P < 0.05$) and tracheal-obstructed rats (8.3 ± 0.8 sec to 6.1 ± 0.9 sec) (df = 9, t = 3.38, $P < 0.01$) (paired t-test). This phenomenon was also observed 4 weeks after surgery in all groups (data not shown). The escape latencies decreased gradually during the active avoidance tests indicating that the rats learned to escape from foot shock and responded more rapidly as the trials progressed (Fig. 3). At week 2 after surgery, the number of avoidance responses in the first 10 trials was not different between the sham-operated rats and tracheal-obstructed rats. However, when the test was performed at week 4 after surgery, tracheal obstruction significantly decreased the number of avoidance responses in the first 10 trials compared to sham-operated rats [$F(3,84) = 2.68$, $P < 0.05$] and this effect was inhibited in tracheal-obstructed rats receiving surgical relief on week 2, but not on week 3 (Fig. 4A). At week 2

Table 3. Lack of effect of tracheal obstruction on behavior in the elevated plus-maze test

	Week 2 Groups		Week 4 Groups			
	Sham Operation (n = 9)	Tracheal Obstruction (n = 10)	Sham Operation (n = 22)	Tracheal Obstruction (n = 18)	Tracheal Obstruction + Surgical Relief on Week 2 (n = 22)	Tracheal Obstruction + Surgical Relief on Week 3 (n = 23)
Open arm time (sec)	5.4 ± 3.5	21.8 ± 11.3	27.5 ± 10.2	23.7 ± 6.9	17.8 ± 6.2	14.7 ± 4.7
Enclosed arm time (sec)	265.9 ± 5.5	235.6 ± 17.9	238.0 ± 13.1	250.5 ± 9.1	255.0 ± 10.5	261.7 ± 7.1
Open arm entry (no.)	0.7 ± 0.4	2.4 ± 1.2	2.6 ± 0.6	3.4 ± 0.8	2.1 ± 0.6	2.1 ± 0.6
Enclosed arm entry (no.)	6.2 ± 1.0	6.0 ± 0.9	8.6 ± 1.0	8.6 ± 1.5	7.4 ± 1.1	7.9 ± 1.0
Risk assessment (no.)	1.1 ± 0.5	2.3 ± 0.9	1.6 ± 0.2	1.4 ± 0.3	1.0 ± 0.2	1.7 ± 0.5

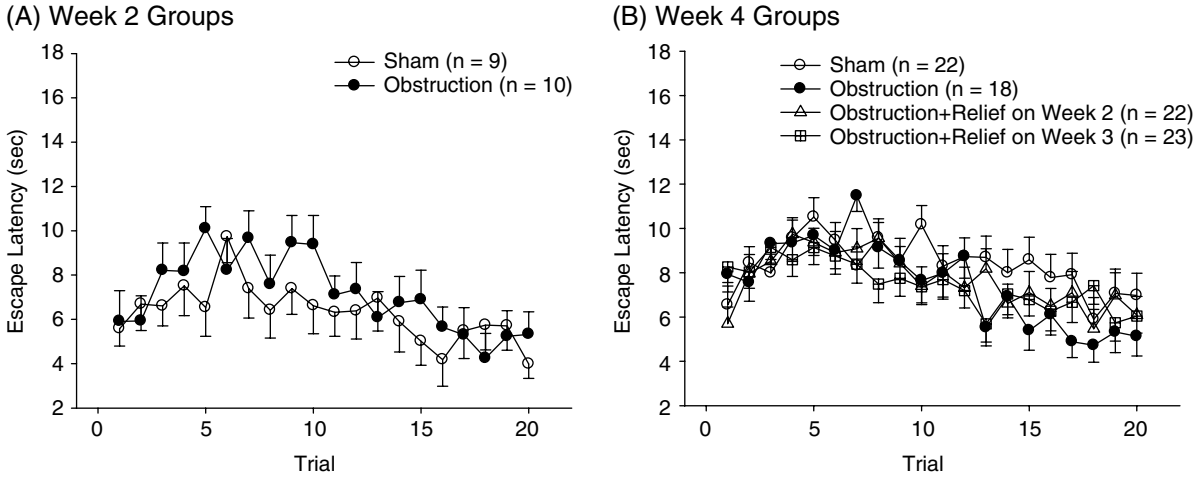

Fig. 3. Lack of effect of tracheal obstruction on the escape latency in the active avoidance test. The test was performed 2 (A) or 4 weeks (B) after tracheal obstruction. The data are means ± SEM.

or 4 after surgery, the numbers of escape responses and failure responses were not different between the sham-operated and tracheal-obstructed rats, irrespective of whether or not they received surgical relief (Fig. 4, B and C).

Discussion

In this study, we established a rat model of tracheal obstruction, evaluated its physical and behavioral consequence, and demonstrated the benefits of surgical intervention.

Learning impairments were observed following tracheal obstruction, and early relief of the obstruction prevented these deficits. Immediately after tracheal obstruction, the respiratory rate decreased and the difference in esophageal pressure increased indicating an enhancement of resistive airway loading. This obstruction seemed not to cause hypoxia, as blood gases were not altered. Moreover, body weight gain was not affected at 2 or 4 weeks after tracheal obstruction. In the current study, emotional anxiety was not affected by tracheal obstruction, as open arm time and risk assessment in the EPM test were not changed. In addition, the motor activity of the rats, as indicated by the total number of arm entries in the EPM test (30), was also not affected. The active avoidance test data demonstrated that the escape latency decreased gradually across trials and that the number of avoidance responses was higher during trials 11-20 than during trials 1-10 suggesting that the rats showed learning behavior in this test. However, the learning efficiency, indicated by the number of avoidance responses in the first 10 trials (17), was impaired in rats after 4 weeks, but not after 2 weeks of tracheal obstruction, and this learning impairment was prevented by surgical relief of tracheal obstruction performed on week 2, but not when performed on week 3. These results suggest that chronic tracheal obstruction causes physical and learning dysfunctions and that early surgical intervention is necessary to prevent potential detrimental effects of tracheal obstruction.

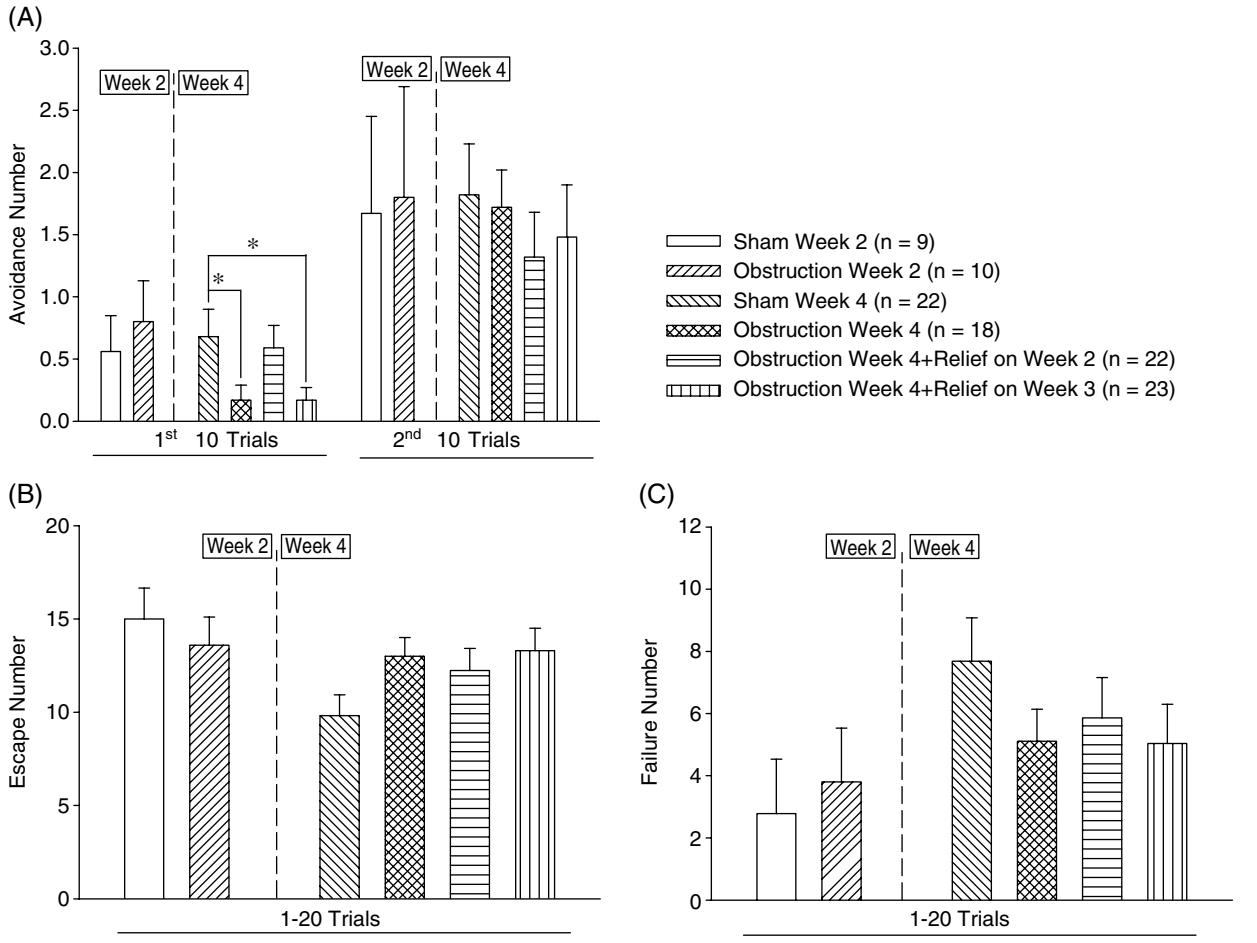

Fig. 4. Effect of tracheal obstruction on behavior in the active avoidance test. The test was performed 2 (A) or 4 weeks (B) after tracheal obstruction. The data are means ± SEM. $*P < 0.05$ compared to the sham-operated group.

A previous study has demonstrated that school-aged children with undiagnosed or untreated CVR show lowered intellectual capabilities than age-matched controls (2), which were reversed after surgical intervention (8). Similarly, tracheal obstruction-induced learning impairment was prevented by surgical relief of the obstruction. In line with a previous report showing that CVR did not cause significant hypoxemia (10), the current data revealed that 4 weeks after tracheal obstruction, no differences in the concentration of arterial blood gases were observed between tracheal-obstructed rats and sham-operated rats suggesting that hypoxia may not be an important factor in the behavior and mental dysfunction seen in CVR.

In the active avoidance test, rats can escape from the aversive shock by crossing to the opposite compartment of the shuttle box. Over a number of trials, the rats can learn to make a shuttling response in order to avoid or escape from the electrical stimulus (13, 17). In the present study, the rats manifested a gradual increase in speed of response to the CS and UCS, thus showing decreasing of escape latency. This phenomenon was much clearer in the second tier of trials where, in addition, the avoidance number was higher than that in the first 10 trials and revealed no between-group difference indicating that the rats had learned an appropriate response to the pairing of CS and UCS. Since escape latency and number of avoidance in the test are indices of learning acquisition (14), the difference of avoidance number in the first tier of trials has been demonstrated as an indication of learning efficacy, namely, the rats with high learning efficiency, compared to low efficient rats, showed higher avoidance number in the first half of the test (17). The rats receiving 4 weeks of tracheal obstruction showed less number of avoidance responses in the first 10 trials of the active avoidance test compared to the sham-operated group indicating a deficit in learning efficacy. The deficit of performance in the active avoidance test was not due to nonspecific effects on motor activity, as the total number of arm entries in the EPM test was not affected.

Our previous study showed that anxiety levels

can affect behavioral performance in the active avoidance test and that rats with low anxiety levels learn to avoid the foot shock quicker than high anxiety rats (17), consistent with the observation that anxiolytic manipulations can improve active avoidance performance (12, 29). However, the anxiety levels of the rats were not affected by tracheal obstruction or relief surgery. Pain sensation has been reported not to be involved in the behavior response in the inhibitory avoidance test (6). Although the pain threshold of the rats was not measured in the present study, struggling and vocalization were observed during the foot shock indicating intact pain sensation. Moreover, CVR is a group of congenital aortic arch abnormalities that is normally diagnosed in early infancy (5, 21). Vascular ring-induced symptoms are normally observed in infants and young children (25), but some complications are observed in adults (20) and even in middle-aged people (15). Similarly, our results revealed behavioral impairments caused by tracheal obstruction in adult animals. It is noted that tracheal obstruction performed on neonatal or young animals may provide further data for elucidating impacts on neuronal and behavioral development. Further, patients with upper airway resistance syndrome often present sleep-disordered breathing that has been known to be associated with neurological dysfunction and even psychological symptoms (1). Thus, further studies on the correlation between sleep-disordered breathing and behavioral changes in tracheal-obstructed rats will provide data for elucidating the cause of cognitive impairments in CVR.

Based on three dimensions of predictive, face and construct validities that are used to assess the validity of animal models (32), we suggest that rats that undergo tracheal obstruction may be a model for CVR because they fulfill, at least in part, face and predictive validities as that respiratory symptoms (9) and learning impairments are observed in both the model and patients with CVR (2) and that early surgical management is an effective intervention in this model and in clinical practice (8, 9, 25).

In conclusion, results of this study suggest that chronic resistive airway loading can cause learning impairment similar to observations in patients with tracheal obstruction caused by CVR. The impairment was prevented in rats receiving early surgical relief, but delayed treatment had no beneficial effect indicating the importance of early diagnosis and intervention. Given the present findings in this rat model of airway obstruction, further investigation of the impact of tracheal obstruction on neuronal and cognitive functions, in addition to the traditional focus on respiratory difficulty, is warranted in order to develop better diagnosis and treatment to prevent possible disabilities in these patients.

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