

Exposure chamber for allergen challenge

The development and validation of a new concept

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Exposure chambers have proven to be valuable tools in studying allergic diseases. The chamber provides a controlled environment and maintains conditions for measuring the amount of allergen inducing symptoms in allergic subjects. The aim of the present study was to develop and test an exposure chamber. The chamber was constructed as an airtight tent, made of transparent polyethylene, easy to adapt to the shape of an existing room, easy to clean, and providing exact allergen-dosage control. Airflow to the interior of the tent was controlled by a variable inlet ventilator fitted with a micropore filter and balanced by a variable high-volume air-sampler on the outlet side. Trace material and allergen were administered as aerosols with a nebulizer connected to the inlet pipe. Samples were obtained from interior surfaces and filters at the outlet. Two different methods were used to test the concept. One method used a colored, neutral trace substance (phenol red indicator) measured photometrically on extracts from filters. Secondly, house-dust mite allergen (*Dermatophagoides pteronyssinus*) was applied, with samples analyzed by an ELISA technique. The results demonstrated the ability of the system to administer and sample allergen with a high degree of reproducibility. A clinical pilot trial proved the capability of the system to initiate symptoms in allergic subjects.

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Exposure chambers allow exposure with a well-defined allergen at a low concentration for an extended period of time, while monitoring the trial subject. In this respect, exposure chamber models mimic natural circumstances, and make it possible to establish the threshold level of the allergen used.

Previous studies have described the use of exposure chambers in establishing the dose needed to provoke symptoms in the allergic subject. In these trials, aeroallergens from cat and pollen, or fecal particles from house-dust mites have been used, and various chambers and methods for distributing the allergens applied (1–6). The exposure-chamber model has also proven valuable in trials comparing the effect and time of onset of antihistamines at a known allergenic load (4, 5).

The purpose of the present study was to develop, construct, and validate a new concept of inhalation provocation. Our goal was a system that is easy to establish; that uses standard equipment for ventilation, allergen distribution, and sampling; that is easy to clean, i.e., eliminating residual allergens; and that is easy to adapt to the shape of an existing room.

Material and methods

Exposure chamber

The exposure chamber was built as an airtight tent (August Olsens Eft. Tent Construction, Copenhagen, Denmark) covering a floor area of 5 m² and having a maximum internal height of 2.6 m (volume: 11.8 m³) (Fig. 1). Polyethylene (PE) (Polysheet Inc., Herlev, Denmark) was chosen as the material for the tent construction, because it demonstrated a good ability to stay antistatic. The PE used for the walls of the tent was transparent, had a thickness of 0.8 mm, and was reinforced with an internal net to provide sufficient strength. The floor material was black PE (thickness: 1.2 mm). Stability was obtained by externally mounted elastic strings in all corners, fixed to the ceiling in the room housing the system. The PE sheets were welded together, the weldings internally sealed by silicone to create a smooth surface. Cables and tubes for internal equipment were led through the tent walls in silicone-sealed guide hoses. The entrance was

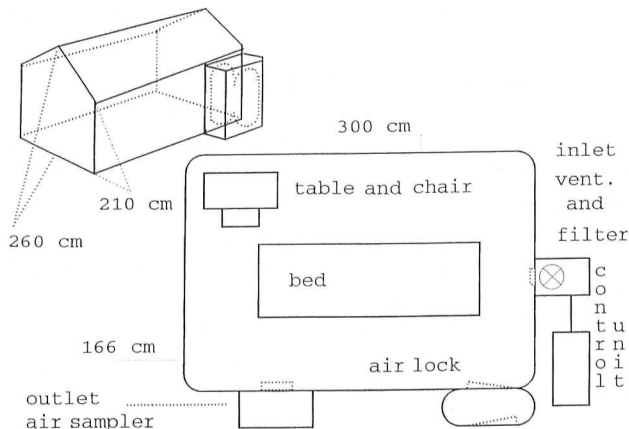


Fig. 1. Exposure chamber: proportions and equipment.

through an air lock, equipped with double boat zip fasteners.

Aerosols

The aerosols used in the system were generated by a standard Porta-Neb[®] 50 medical air compression pump (Medic Aid Ltd, Pagham, West Sussex, UK) generating a flow of 6.5 l/min at 14 psi (0.97 bar). The device was mounted outside the chamber and supplied air to a disposable nebulizing chamber, System 22[®] ACORN (Medic Aid, UK). The mean mass diameter of the aerosol particles produced in comparable setups was 4.1 μ m (7). The aerosol was delivered into the air pumped into the chamber by the low-voltage ventilator (Fig. 1). The nebulizer was used in 10-min sequences, separated by 2-min intervals, as pilot studies had demonstrated that intermittent dosing reduced the residual volume in the nebulizer. Two or four nebulizing sequences initiated all experiments, i.e., a 10-min sequence per milliliter of fill in the nebulizer. Before and after all experiments, the nebulizing chamber was weighed, and the concentration of test substance in the residual volume was measured, in accordance with the changes described in previous studies of aerosols (8, 9).

Ventilation and air-sampling equipment

Air was pumped into the chamber by a low-voltage, variable-speed motor centrifugal fan, Papst Multifan 3312 (Papst Motoren, St Georgen, Germany)[®], 4–16 V DC, fed through a 100-cm² interchangeable, multilayer filter with 20- μ m pore size (Viledon[®] micropore filters, Germany). Power to the inlet ventilator was delivered by a low-voltage DC power supply, B & O[®] SN14 (Bang and Olufsen, Struer, Denmark). Air outlet was through a Sartorius[®] MD 8 high-volume air-sampler (Sartorius GmbH, Göttingen, Germany).

The outlet was fitted with an 80-mm-diameter glass EPM 2000 microfiber filter (Whatman[®], Maidstone, UK), with a pore size of 0.6 μ m, which retained the particles (trace aerosols or aeroallergens) to be quantified (4, 10). An air-volume flow range of 4–6000 l/h was used in all experiments, giving an air exchange of 0.34–0.5/h. In the colored aerosol experiments, a portable air-sampling unit was carried by the trial subject (see below). This unit was a low-volume SKC[®] air-sampler, model 224 PCXR7 (SKC Inc., Eighty-Four, PA, USA) with a 25-mm-diameter glass microfiber EPM 2000 filter (pore size: 0.6 μ m). Sampling was performed for 200 min at 4.0 l/min. Airflow was balanced in this controlled environment by regulating the low-voltage power supply for the inlet ventilator. A static internal air pressure was obtained by observing the tension of the tent walls, and adjusting the inlet flow rate. A difference between inside and outside pressure of ± 1 millibar (1 hPa) was clearly visible in the tension of the tent wall and could be adjusted. Measurements were made at ambient air humidity (range: 30–52% r.h.) and temperature (range: 20–27°C). These parameters were continuously monitored in the allergen experiments.

Air mixing was maintained by having the trial subject follow an occupancy pattern with standardized movements with 2 min of activity every 10 min to ensure reproducible air movement during experiments (Table 1).

Extraction of material from air-sampler filters

The methods developed by Johnsen et al. were used in handling the material obtained from the air-samplers (11). The glass microfiber filters removed from the air-samplers were folded twice, enclosed in vellum envelopes, and crushed in a hydraulic press (500 kg/cm²) (Combac 10, Juelsminde, Denmark). The powdered filter material was transferred to 10-ml polypropylene test tubes and suspended in 2.0-ml modified Sørensen buffer (PBS). The Sørensen phosphate buffer 1/15 M, pH 7.0, was modified by adding sodium azide (200 mg/l), Tween 20 (0.5% v/v), and ϵ -amino-*n*-caproic acid

Table 1. Schedule for standardized movement inside exposure chamber. All activities were performed for 2 min. Between periods of activity, trial subject remained seated. Schedule was repeated every hour

Time (min)	Activity
0	Making bed
10	Walking
20	Adjusting pillow
30	Walking
40	Making bed
50	Walking

(0.1 mol/l). Extraction continued by vertical rotation of the test tubes overnight at room temperature before centrifugation at 1850 g for 15 min. The supernatants were used for measurements.

Sampling from internal surfaces and cleaning methods

After all experiments, samples were collected from the center of the four walls, the ceiling, and the floor. These samples were obtained by using small pieces (6 × 6 cm) of synthetic chamois leather (Dufty, Glostrup, Denmark), each moistened with 2.00 ml PBS. One square meter of all internal surfaces was wiped. The fluid was subsequently recovered from the cloth pieces by mechanical squeezing (40 kg/cm²) in 20-ml syringes.

Various schedules were tested in an effort to develop an accurate and standardized method to clean the interior surfaces and to ensure that a minimum of test material was left on the walls after each experiment. The method chosen involved wet cleaning for 20 min, followed by 2 h of drying, using the ventilation in the closed chamber. This procedure was performed twice. Various cleaning solutions for domestic use were tried, and a product offering good cleaning ability, as proved in repeated standardized cleaning sessions, was chosen (Ajax Original, Colgate-Palmolive, Glostrup, Denmark). For monitoring of residual levels of test substances, both wall samples and air samples were obtained after cleaning was performed, and analysis showed levels below detection limit, i.e., 0.1% of the total maximum dose used in the allergen series. After each cleaning session, the interior was treated with an antistatic solution (Burnus Kunststoff Reiniger) (Burnus GmbH, Darmstadt, Germany).

Test substances

Colored solution. To define the aerodynamic properties of the challenge chamber and the effect of human activity inside the chamber, experiments were performed, using colored solution as a model for allergen extract. Phenol red (BDH Chemicals Ltd, Poole, UK) 1.25 mg/ml, diluted in 2.00 ml of PBS, was chosen as a biologically inert tracer substance for nebulization. The colored supernatants from filters and internal surfaces, obtained as described above, were analyzed in duplicate photometrically in an ELISA reader (NUNC® TIM 200) (NUNC, Roskilde, Denmark) using a 550-nm filter.

Allergen extract. The allergen extract used in the study was a purified intermediary product made from a culture of the house-dust mite *Dermatophagoides pteronyssinus* (*Der p*), kindly pro-

vided by Carsten Schou, PhD, ALK, Hørsholm, Denmark. Aerosols were made with an amount of allergen equivalent to a total of 600–1800 ng of the major allergen *Der p* I, i.e., concentrations of 150–450 ng/ml of fill in the nebulizer. Allergen solutions were made with diluent containing 0.03% human serum albumin, 0.5% sodium chloride, 0.25% sodium hydrogen carbonate, and 0.5% phenol in aqueous solution (Aquagen® diluent, ALK, Hørsholm, Denmark). In the allergen experiments, 4.00 ml of this solution was used in the nebulizer. Immunochemical analysis was undertaken with a commercially available ELISA technique, ALK Indoor Allergen Analysis Kit®, according to the manufacturer's instructions. In brief, test samples were incubated for 2 h in microwell plates precoated with antibody against *Der p* I allergen. Unbound material was washed away and horseradish peroxidase (HRP)-conjugated antibody was added. After incubation for a further 1 h and washing, a chromogenic substrate for the HRP was added. The color reaction was stopped by adding a stop buffer. The microwell plates were measured on an automatic photometer (NUNC® TIM 200) using a 490-nm filter. All samples were analyzed in duplicate. The detection limit was 2 ng *Der p* I per ml.

Clinical pilot trial

To assess the clinical properties of our chamber concept and validate the dose of mite allergen applied, we performed an open study. This pilot trial involved three volunteer asthmatic subjects with proven allergy to house-dust mite. The three patients were all male (ages: A=20, B=26, and C=23 years). Allergy was confirmed by positive skin prick test to house-dust mite allergen *Der p* (ALK Soluprick) – diameter > 3 mm, a 1-mm lancet being used (5). Allergen-specific IgE was shown to be present by positive Magic Lite® (ALK) test to *Der p* ≥ class 2 and positive bronchial challenge test, PD₂₀ (≥ 20% drop in FEV₁ after maximum 100 000 SQ-U) (ALK) *Der p* dosage) with a conventional nebulizing method, as described elsewhere (12). All subjects suffered from perennial asthma and showed a reversibility of at least 15% in FEV₁ after β₂-agonist inhalation. Diaries were kept and trials in the exposure chamber were performed twice after a stable period of at least 14 days with a variation in PEFR of less than 15%. Diaries were continued for 14 days after the second trial to ensure normalization and monitor late reactions. The patients stayed in the challenge chamber for 2–3 h. Local steroid treatment was stopped for 48 h, and β₂-agonist for 12 h before the challenges. An allergen extract equivalent of 600 or 1200 ng *Der p* I in 4 ml of the diluent mentioned above was

used. A theoretic maximal concentration of 50 or 100 ng/m³ was achieved in this manner if instantaneous, even distribution was assumed. The dosage was chosen in view of the findings of Sakagushi et al. (13) and Horak et al. (4) and was based on the losses in the nebulizer encountered in the allergen experiments mentioned above. In the chamber, the patients recorded symptoms from the eyes, the nose, the skin, and other organs. Symptoms were scored on a scale of 0–3 (no symptoms=0; 3=heavy symptoms). Measurements of PEF_R, FEV₁, and FVC were made every 15 min. After the patients had left the chamber, continuous monitoring took place for 1–3 h.

Statistics

Nonparametric statistics were applied to test the paired and unpaired data in the two series of experiments (Mann-Whitney U test). Two-tailed *P* values of <0.05 were considered significant. The computer software used was SPSS/PC+, Version 4.0 (SPSS Inc., Chicago, IL, USA).

Results

Experiments with colored aerosol

Fig. 2 presents the results from the colored aerosol experiments as percentages of the total amount of test substance delivered, corrected for residual amount in the nebulizer, and of test substance

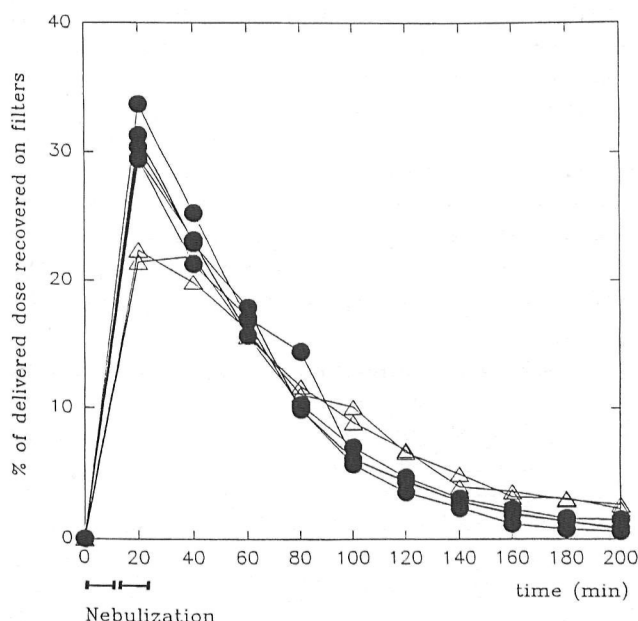


Fig. 2. Amounts of phenol red trace substance on air-sampler filters, percentage of total amount recovered. Filters were changed every 20 min. Nebulizer was used for total of 20 min (two periods of 10 min separated by 2-min interval) in initiating experiments. Standardized activity: ●; no activity: △.

recovered from internal surfaces. Activity in the exposure chamber increased the initial peak amount on filters and increased the subsequent elimination rate. Within 40 min, more material, mean 54% (range: 51–59%), was collected on the air-sampler filter when the air was disturbed by activity than when there was no activity: 43% (range: 42–43%). The five curves representing the data from the experiments with standardized activity presented a steeper slope initially, and a more rapid decline after the peak reached within 20 min. The set of curves which represents the experiments without human activity reached a lower peak and demonstrated a slower decline as the two different sets of curves crossed. However, the differences were only significantly different at the 100-, 140-, and 180-min measurements (*P*<0.05) and only when corrections for ties were performed in the statistics test. The area below the curve under the two different circumstances was not significantly different.

Samples from the low-volume SKC[®] air-sampler showed values of test substance less than 1% of the total distributed amount.

The amounts settled on internal surfaces during the experiments accounted for 5% (range 0.5–8%) of the total dose delivered to the system, when extrapolating from the measured surfaces. The reproducibility of the delivery is illustrated by Fig. 2, with a coefficient of variation below 0.05 at any point. The mean concentration of test substance in the residual volume in the nebulizer after each experiment increased 2.1-fold (range: 1.9–2.1). The residual volumes represented a mean of 9% (range 6–17%) of the initial volume present in the nebulizer.

Allergen aerosol experiments

Fig. 3 illustrates the accumulated amounts of house-dust mite allergen on the filters of the air-sampling unit during experiments, expressed as percentages of the total amount of allergen recovered on the air-sampler filters. The absence of human activity in the chamber again led to a different behavior of the aerosol delivered. The coefficient of variation was higher than in the colored aerosol experiments: maximum 0.18 at 45 min. A mean of 57% (range: 51–64%) was recovered from the system within 1 h under circumstances with activity, versus 37% (range: 35–39%) without activity (*P*=0.025). The amounts of allergen recovered in the experiments without human activity in the chamber were significantly lower in all the measurements (*P*<0.05). The mean concentration of the residual allergen solution in the nebulizer after each experiment increased 1.9-fold (1.85–1.93). The mean residual

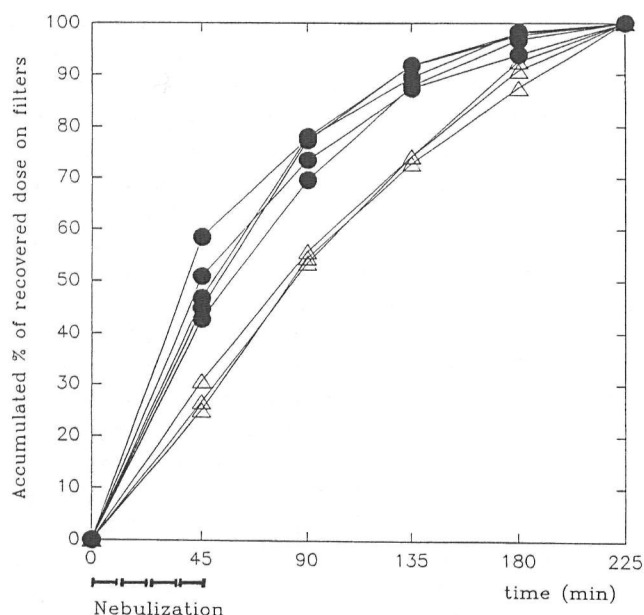


Fig. 3. Accumulated amounts of allergen (percentages) recovered on air-sampler filters. Filters were changed every 45 min. Nebulizer was used for 40 min in initiating experiments (four periods of 10 min separated by 2-min intervals). Standardized activity: ●; no activity: △.

volume represented 8% (range: 5–16%) of the initial volume in the nebulizer in these experiments.

Allergen amounts above the 2-ng cutoff level, as defined in the ELISA technique, were not found on any examined square meter of internal wall surface in any of the experiments.

Results from clinical pilot trial

The three patients included in the clinical pilot trial all reacted with various symptoms to the allergen challenge (Table 2).

Discussion

House-dust mite allergens are a common cause of perennial allergy in many geographic regions (9,

10, 13, 14). For allergens with moderate seasonal variation, i.e., house-dust mites, molds, or mammals, the case history alone cannot always confirm the clinical suspicion raised by skin or *in vitro* testing. A precise diagnosis is essential when one is considering change in occupation, or major change in the home environment, such as removal of furniture and animals or change in residence. Under these circumstances, allergen challenge in the target organ may sometimes be necessary to secure a high level of diagnostic certainty. However, the methods currently used for bronchial allergen challenge introduce high peak levels of allergen directly to the shock organ, thus, in some instances, causing instant appearance of symptoms, immediate discomfort, and often late-phase reactions. These risks have restricted bronchial challenges, as currently conducted, mainly to investigation of subjects in whom occupational allergy is suspected, or to research purposes.

Exposure chambers have been introduced as a more convenient tool to provide a controlled allergen exposure using allergen at "physiologic" levels. Previous studies have entailed rather costly practical solutions; e.g., converting existing rooms to controlled environments for challenge tests (1, 2, 5). To offer a more affordable and flexible solution, our chamber concept used standard components for nebulization, ventilation, and air-sampling. The chamber itself was a synthetic polyethylene tent, easily adaptable to an existing room. Adhesion of allergen to internal wall surfaces was minimized by antistatic treatment, as proven by the recovery of only small amounts of test substances on the internal surfaces after experiments. The chamber described in this paper was designed and constructed to use house-dust mite allergen in nebulized form as allergen source. The mean diameter of particles containing house-dust mite allergens under natural exposure is large; a mean value of 20 μm (10, 15). This causes the main proportion of the house-dust mite allergen particles to become air-

Table 2. Results from clinical pilot trial. PD₂₀: House-dust mite allergen dosage needed to give 20% drop in FEV₁ in traditional challenge. Interpolation was performed in semilogarithmic system

Patient and visit no.	PD ₂₀ (SQ-U)	Total amount of Der p I used in exposure chamber (ng)	Decrease in FEV ₁ (%)	Interval from start to maximum decrease in FEV ₁ (min)	Comments
A1	7000	600	14	90	Symptoms (eyes and nose) after 15 min
2		600	8	135	Symptoms (nose) after 15 min
3		1200	57	30	
B1	8000	600	7	105	Cough after 60 min
2		600	7	60	Itching of skin after 15 min
C1	55 000	600	10	60	Nasal obstruction after 120 min
2		600	13	90	

borne only briefly after disturbance (15–30 min), unlike other aeroallergens such as cat allergen (5, 6, 10, 16). Some of the large particles carrying house-dust mite allergen disintegrate and allergen appears on smaller particles of only a few micrometers, the amount of this fractionation probably being dependent on the humidity, as exsiccation seems to accelerate fractionation of the particles. Thus, for examination of threshold levels of airborne house-dust mite allergens in an exposure-chamber model, we have applied an aerosol method, providing standardized particle size and ensuring that the allergen stays airborne long enough to induce symptoms and enable allergen quantification. The use of initial nebulization mimics the natural profile of exposure with peak levels of allergen after disturbance (6, 11, 17–19), because of the natural aerodynamic behavior of the particles carrying house-dust mite allergen, as described previously (16). Horak et al. used a constant allergenic load of house-dust mite fecal particles pumped into the air in the Vienna challenge chamber. When using this model in an 8-h challenge, they found that the ocular and nasal symptoms observed increased initially (within the first hour) before reaching a plateau, while bronchial reactivity was biphasic with both an initial and a late-phase reaction (4).

It is still an open question whether the intensity of the reactions in allergic subjects is determined by the peak allergen level, or by the accumulated dose of allergen; however, the aerodynamic properties of different aeroallergens show great variation, as demonstrated in previous studies (12, 15, 16, 20, 21). In view of these differences, the need for improved challenge methods in exposure-chamber models, depending on the allergens chosen, becomes apparent.

The system designed and built for our study has been shown to provide the properties demanded of a controlled environment suited for house-dust mite aeroallergen challenge.

The kinetics of the produced aerosols showed an initial peak level, reached within 1 h, followed by a slow decline in the allergenic load. The reproducibility was high, and the ability to minimize the internal residuals of test substance in the system was good. The impact of controlled movements, i.e., turbulent airflow in the exposure chamber, was illustrated. Human activity changed the kinetics. Comparison of the kinetics of the phenol red aerosols and the allergen aerosols showed a difference between the aerodynamic properties of the two solutions. Variations in molecular mass and protein content could explain this difference, as described in former studies of aerosols (8, 22). A residual volume with high allergen concentration left in the nebulizer

could be kept at a constant level when a standardized schedule for nebulization was followed (18).

Only very small amounts, less than 1% of the total recovered phenol red dose, were found on the filters of the low-volume air-sampler unit. Theoretically, the total volume of 800 l of air pumped through the filter should have resulted in larger settled amounts, i.e., 4%, equal to 70 ng, well above the detection limit of 2 ng. These results led to the conclusion that sampling with this unit was of limited value; consequently, the low-volume sampler was not used in the allergen series. The problem might be caused by adhesion to the outside of the filter-holding unit. Reshaping the filter-holding unit of this equipment could be the subject for additional investigation in our exposure chamber.

The allergen dosages used in the clinical pilot trial were chosen in light of the previous work of Sakagushi et al., who found concentrations of *Der p* I of 0.03–30 ng m³ air in different houses (13), and Horak et al., who could induce clinical symptoms in the nose at a constant allergenic load of 40 ng/m³ air (4). In the present study, a theoretical peak concentration of 50 or 100 ng/m³ air was achieved if instantaneous, even distribution was assumed. This proved to be sufficient to cause clinical symptoms in the three allergic subjects tested. As the dosage varies as an effect of air-sampling and distribution, the actual dose is lower, probably 60–80% of the peak load. In our opinion, this dynamic load simulates natural exposure convincingly. A larger-scale, placebo-controlled, double-blinded study is now in progress to evaluate the concept further.

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