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## The Hot Air Blast as Aversive Stimulus in Escape Contingencies with Rats

O Jato de Ar Quente Como um Estímulo Aversivo em Contingências de Fuga em Ratos

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## Resumo

Com o objetivo de ampliar o número de estímulos aversivos disponíveis para pesquisa em laboratório, testou-se um Jato de Ar Quente (JAQ) como reforcador negativo em duas condições de fuga utilizando ratos como sujeitos. No Experimento 1 a resposta de fuga "saltar" eliminava o evento aversivo. No Experimento 2 a resposta de fuga "focinhar" eliminava o evento aversivo. Em ambos os estudos, 7 de 8 indivíduos (87,5%) testados em cada condição aprenderam a resposta de fuga necessária ("saltar" e "focinhar", respectivamente). Os dois estudos atestaram a função reforçadora negativa do JAQ em contexto de fuga e, assim, oferecem uma nova alternativa de evento aversivo a ser adotado em pesquisas nessa área. Palavras-chave: fuga; reforçamento negativo; jato de ar quente; controle aversivo.

## Abstract

To increase the number of aversive stimuli that are available for laboratory research, the hot air blast (HAB) was tested as a negative reinforcer in two escape contingencies. Sixteen naïve rats were exposed to 30 or 60 HAB presentations. For half of the subjects, the escape response was jumping in a shuttle box; for the others, the HAB was interrupted after a nose poke response. The results showed that seven of eight subjects (87.5%) in each group learned the required escape response. These data confirm the negative reinforcing function of the HAB, which may be an alternative aversive stimulus to be adopted in research with nonhumans subjects.

Keywords: escape; negative reinforcement; hot air blast; aversive control.

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Most laboratory studies that investigate the effects of aversive events on responding have adopted electric shocks as the punisher or negative reinforcer (Azrin & Holz, 1966/1975, p. 463; Baron, 1991, p. 176; Domjan & Burkhard, 1993, p. 283). The principles that have been established for aversive contingencies are largely supported by the presumed generality of effects that are observed with this particular stimulus. However, electric shocks have peculiar physical characteristics and elicit very specific responses (Catania, 1998/1999). Thus, it may not be possible to extend data that are generated with electric shocks to other types of aversive stimuli. Therefore, the generality of established principles of aversive stimuli.

To become useful under laboratory conditions, a stimulus needs to be standardized and reliable under a series of manipulation conditions so that systematic replication is possible. From an ethical perspective, the aversive stimulus should also cause minimal discomfort and organic/behavioral sequelae for experimental subjects. They should also maintain a sufficiently aversive function from a scientific point of view and be efficient for experimental studies of several parameters and behavioral measures (Azrin & Holz, 1966/1975). All these requirements considerably restrict the expansion of studies that utilize other stimuli than electric shocks.

Few stimuli, besides electric shock, were used as an aversive stimulus in behavioral analytic research. For example, in early studies developed by Thorndike (1898), basically two aversive stimuli were used. In studies with cats, dogs, and monkeys, containment in the box itself (i.e., the restriction of physical space) served as the aversive stimulus, which was automatically eliminated by leaving the apparatus as the escape response. In studies with fish, the light was used as the aversive stimulus: to swim to the dark side of the aquarium was the escape response. Later, Skinner (1938) used a device that slapped the paws of mice when they pressed a lever, reducing the frequency of these responses (punishment). Subsequent studies used sound as the aversive stimulus for human subjects (e.g., Azrin, 1958; Herman & Azrin, 1964), which has also been used with pigeons (Holz & Azrin, 1962), cats (Barry Jr. & Degelman, 1961), and rats (Riess, 1970; Knutson & Bailey, 1974). Other stimuli have been used, albeit with a lower frequency, such as luminous intensity in rats (F. S. Keller, 1941; J. V. Keller, 1966), nausea-inducing food using x-rays in rats (Garcia & Koelling, 1966; Smith & Roll, 1967), cat odor in rats (Hubbard et al., 2004), wind bursts in monkeys (Rohles Jr., 1965), and pressurized air puffs in rats (Ray Jr., 1966; Ray Jr. & Lenz, 1968; Clark, Vasilevsky & Myers, 2003; Myers, Cohn, & Clark, 2005) and monkeys (Baker & Ziegelbauer, 1969).

We recently sought to identify an alternative aversive stimulus other than electric shocks that is different from the aforementioned stimuli and can be employed at a low cost with regard to equipment construction. We employed a hot air blast (HAB). Our initial data (Carvalho Neto et al. 2005; Carvalho Neto, Maestri, & Menezes, 2007) showed that the HAB suppresses responding in Rattus norvegicus exposed to punishment contingencies. In these two preliminary studies, the lever-press response was previously reinforced on a continuous reinforcement (CRF) schedule with a positive reinforcer, and this schedule was maintained during the punishment phase. Superimposed on the positive CRF schedule, the HAB was presented, contingent on a lever press in two different schedules for each group: continuous punishment (CRF schedule) and intermittent punishment (fixed-ratio 3 schedule). Carvalho Neto et al (2005) reported that the levels of response suppression found after two sessions of punishment were 98.4% (CRF schedule) and 71.1% (FR3 schedule). In the second study, Carvalho Neto et al. (2007) manipulated the number of punishment sessions along with two experiments. In the first one, after 10 punishment sessions the response suppression level obtained was 86.2% (CRF) and 50.2% (FR3); in the second experiment, after 20 punishment sessions the response suppression level obtained was 92.2% and 57.9%, for CRF and FR3 schedules respectively. Such studies indicate that the HAB has a suppressing aversive function over-responding (positive punishment), and such a function can be maintained even after 20 sessions. More recent data confirmed the suppressive effects of HAB in animals with different positive reinforcement histories (Carvalho Neto, Magalhães, Santos, & Mayer, 2018) and in arrangements using contingent and noncontingent aversive presentations (Gaspar, Carvalho Neto, & Maver, 2019).

No experiments have tested the HAB regarding its negative reinforcement function. The studies that come closest to this test showed that pressurized air blasts can have a negative reinforcement function in rats (Clark, Vasilevsky & Myers, 2003; Mayers, Cohn & Clark, 2005; Ray Jr., 1966). However, this stimulus is not equivalent to HAB. In addition, the equipment that is necessary to generate pressurized air blasts is relatively expensive, what can be a reason that makes it difficult to use. Thus, the objective of the present study was to verify if the HAB – produced by inexpensive equipment - can function as a negative reinforcer for rats. The HAB was tested as the negative reinforcer in two escape contingencies by adopting the jump and the nose poke responses as the requirement necessary to eliminate the aversive stimulus.

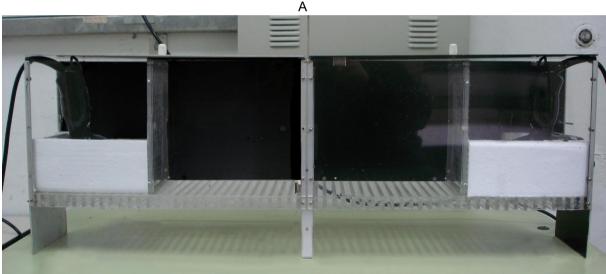
#### Subjects

## Method

The subjects were sixteen female Wistar rats, experimentally naive and ~3 months of age at the beginning of the experiment were obtained from the Butantan Institute (São Paulo, Brazil). The animals weighed ~250 grams at the beginning of the study. The temperature of the vivarium was maintained at ~23°C with 70% humidity and a 12 hour/12-hour light/dark cycle. The study was conducted in 2006 during a postdoctoral internship at the University of São Paulo. The Arouca Law (no. 11.794) that regulated the use of nonhuman animals in research in Brazil was first published in 2008. Therefore, there was no ethics committee on the use of animals (CEUA) at the time of the study. However, all the rules of the former Brazilian College of Animal Experimentation (COBEA) were followed as the ethic procedure.

#### **Apparatus**

A shuttle box (50 cm length, 15.5 cm width, 20 cm height) was adapted for using the HAB in jumping and nose poking escape contingencies. The back wall, ceiling, and partition wall were made of black-painted aluminum. The floor consisted of transparent acrylic bars. For the jumping escape test (Figure 1), the lateral walls were wire screens that allowed the passage of the HAB that emanated from the external two hairdryers on the sides of the box. The partition wall bisected the apparatus into two equally sized compartments, with a passageway from one compartment to the other. A central window (6 cm height, 7.5 cm width) that united the two compartments was 4 cm above the floor. The HABs were triggered from the lateral walls toward the center of the shuttle box at the height of the window. This equipment was an adaptation of that described by Hunziker (1981) as a suitable experimental box for escape investigation with rats.



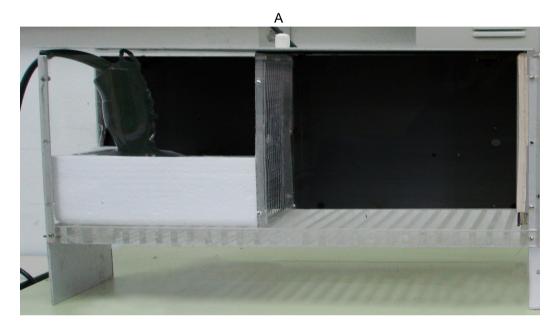
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Figure 1. Shuttlebox adapted for use with the HAB in a jumping escape contingency. (A) Frontal View. (B) Internal view of the partition wall and the central jumping window that connected the two compartments.

For the nose poking escape test, the left side of the shuttle box was isolated and adapted (Figure 2). The central wall where the jumping window was located was closed with a wooden screen. A nose poke hole (2.5 cm diameter) was located in the lower right corner of this screen and touched the floor. An approximately 2 cm space was between the nose poke wall and the original window wall. On the right side of the box above the hole, three 5Watt lamps were installed and remained permanently on, thus producing luminous contrast that emphasized the nose poke hole location. Nosepoking responses were emitted in this hole and manually recorded when the rat's nose was put into the space between the walls. Only the left hairdryer was used during this test.

The HAB was produced by two Revlon hairdryers (model no. RV429AB) that were manually operated. The maximum intensity of the hairdryers was applied in this experiment, which increased the temperature of the air by ~2°C relative to room temperature in 10 seconds over a radius of 10 cm. The air pressure was 216.5 dyn/cm<sup>2</sup>, and the sound level was 85 dB. The sessions were manually controlled, and video recorded.



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Figure 2: Nose poking box adapted for the use of HAB: (A) Frontal view. (B) Amplified internal view: hole for the nose poke response.

### Procedure

The rats were tested under jumping or nose poking escape contingencies.

In the jumping test, the animals were exposed to 30 trials during a single session. Each trial consisted of the presentation of a HAB for a maximum of 10 seconds under a variable time 60 second (VT60) schedule. The HAB was triggered in the compartment where the subject was present. If the rat emitted a jumping

response toward the opposite compartment (without HAB) within 10 seconds, then the HAB was turned off. If the escape response was not emitted within 10 seconds, then the HAB was automatically turned off at the end of this period. In both situations, the trial ended, and a new trial began by continuing the VT60 schedule of presentation of the HAB.

In the nose poking test, the animals were exposed to 60 trials during a single session. Each trial consisted of the presentation of a HAB for a maximum of 10 seconds under a VT60 schedule. The HAB was triggered on the left side of the box. If the rat emitted a nose poke response within 10 seconds, then the HAB was turned off. If the rat did not emit the escape response within 10 seconds, then the HAB was automatically turned off at the end of this period. In both situations, the trial ended, and a new one began by continuing the VT60 schedule of presentation of the HAB.

The usual escape pattern is a feature by the initial high response latency followed by decreasing latencies along with the repeated exposures to the negative reinforcement. The criterion adopted in the present study for measure escape learning was based on visual observation: the mean of the first five latencies (Block 1) was compared with the mean of the last five trials (Block 6 for the jumping test, and Block 12 for the nose poking test). If the final latencies were shorter than the initial ones, the escape learning was registered. If latencies were stable or increased, then this would indicate the absence of learning.

## Results

Figure 3 shows the individual and group data obtained along the jumping escape contingency organized in blocks of 5 trials. The mean group results showed that the rats responded with an initial average latency of ~5 seconds. By the end of the session, the latency decreased to an average of 2 seconds, thus indicating learning during the sessions. Individually, most of the rats produced decreased escape latencies during the session, showing the latency in the final block smaller than at the first block (exception only the Subject 6). So, seven of eight subjects (87.5%) in each group learned the escape response after 30 trials.

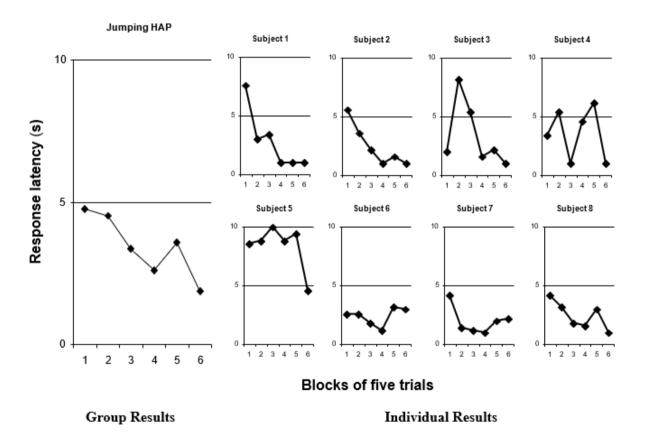


Figure 3: Escape from HAP using jumping as the response. The larger graph on the left shows the average data for the group. The smaller graphs on the right show the individual data.

Figure 4 shows the individual and group data obtained along the nose poking escape contingency organized in blocks of 5 trials. The mean group results showed that the rats responded with an initial average latency of ~8 seconds. By the end of the session, the latency decreased to 5 seconds, which demonstrated

improvements in learning across sessions Seven of eight subjects (87.5%) learned the nose poke escape response after 60 trials: they showed lower escape response latency in the final block comparing with the first one. Only one subject (Subject 7) had longer latencies in the last block.

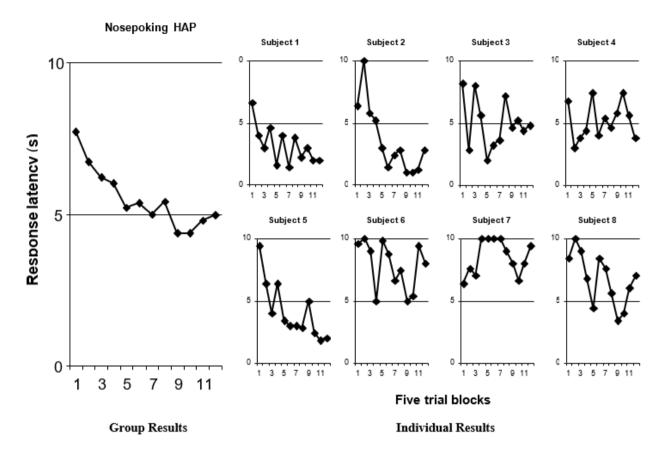


Figure 4: Escape from HAP using nosepoking as response. The larger graph on the left shows the average data for the group. The smaller graphs on the right show the individual data.

### Discussion

The HAB was able to function as a negative reinforcer in an escape contingency using two different types of responses: jumping and nose poking. In both conditions, seven of eight subjects (87.5%) learned the escape response.

The jumping response was more rapidly emitted than the nose poke response, varying from 5 seconds in the initial block to 2 seconds in the final block. The nose poke response varied from 8 seconds in the initial block to 5 seconds in the final block. In the nose poke experiment, higher response variability occurred during the presentation of the HAB, with a higher number of ineffective escape responses, mainly exploratory responses toward the central wall. In the shuttle box, few variabilities were observed, with the more restrictive emission of ineffective escape responses. Such differences would explain the different latencies in each condition.

Phylogenetic differences may be one explanation for the variance observed related to the two responses emission. However, it is difficult to say that this is a biological difference because both jumping and focusing are part of the basic exploratory repertoire of rodents. Nonetheless, the two tasks had some structural differences. In the jumping test, the rat was exposed to an aversive stimulus that was presented on one side of the box, and it had to jump through a central window (6 cm X 7.5 cm) to reach the other compartment. In the nose poking test, the rat had to put its nose in a lighted hole (2.5 cm diameter) that was located in the lower right corner of the wall. The first task would have at least three potential advantages in terms of stimulus discriminability: the size of the window (6.0 x 7.5 cm window with 2.5 cm hole), its height (a window at approximately the same height as the rat's eyes when walking on four paws and a nose poke hole that was below the rats' line of sight), and the possibility of seeing the other side of the box (safe environment). To insert the nose in a certain corner of the box for an escape from an aversive stimulus is a less natural response, for rats, than to jump away from this stimulus.

Despite these differences in average latency in each group (5 to 2 seconds for jumping and 8 to 5 seconds for nose poking), the reduction of latency across sessions was similar (i.e., 3 seconds). However, these data cannot be directly compared because the number of trials were different (30 trials for jumping and 60 trials for nose poking).

We used different numbers of trials in the two tests for practical reasons. Each test was part of a general project whose goal was to identify a new aversive stimulus that can be tested in a learned helplessness model. In such a model, there are traditionally two aversive conditions: treatment and final test. For example, in Hunziker and Santos (2007) experiments, in an initial phase (treatment occurs), the rats were exposed to 60 electric shocks in a nose poking apparatus. The same subjects are then exposed to a final test that consisted of 30 shocks in a shuttle box. In the present study, we replicated the results reported by Hunziker and Santos regarding the naïve escape groups. The results reported here indicate that the HAB and the adapted apparatus we used are useful alternatives for research on escape investigations.

The two experiments contribute to our suggestion that HAB is an aversive stimulus that can be applied under laboratory conditions with negative reinforcing function in escape contingencies. In addition to the scientific contribution of the present findings, the technical contribution is also notable. It is a relatively low-cost apparatus that is highly standardized, and it has readily measurable parameters, indicating that it may be a promising alternative to electric shock or pressurized air blasts.

# **Declaration of conflict of interest**

The authors declare that there is no conflict of interest regarding the publication of this article.

# Contribution of each author

All the authors are equally responsible for the article contents.

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