

ТЕОРІЯ ТА ЕКСПЕРИМЕНТ

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ADAPTIVE PROPERTIES OF *LITSEA CUBEBA* AND *MENTHA PIPERITA* ESSENTIAL OILS AS POTENTIAL AGENTS FOR USE IN AROMATHERAPY OF STRESSOGENIC CONDITIONS

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ЗАХИСНІ ВЛАСТИВОСТІ ЕФІРНИХ ОЛІЙ *LITSEA CUBEBA* ТА *MENTHA PIPERITA* ЯК ПОТЕНЦІЙНИХ ЗАСОБІВ ДЛЯ ВИКОРИСТАННЯ В АРОМАТЕРАПІЇ В СТРЕСОГЕНИХ УМОВАХ

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Вступ. Було здійснено порівняльне вивчення впливу ефірних олій *Mentha piperita* L. та *Litsea cubeba* (Lour.) Pers. на поведінкові реакції та психоемоційний стан мишей у тесті відкритого поля.

Методи. Досліди проводили на безпородних мишах обох статей, які за 1 хв до тестування піддавалися інгаляціям ефірних олій (0,05 мл 1 % розчину гексану) з подальшою реєстрацією поведінкових показників (перетин квадратів периферійної/центральної зони, час перебування в кожній зоні, підйоми на задні лапки), грумінг, акти сечовипускання, дефекації тощо).

Результати. Під впливом ефірної олії *Litsea cubeba* найбільш суттєвий вплив відмічено на показник невідтримуваної орієнтаційної активності (підвищення до 1,5 раза, $p \leq 0,088$), при цьому ефірна олія *Mentha piperita* пригнічує та знижує пошукову активність на 56 %. Інгаляції ефірної олії *Mentha piperita* індукують зниження рівня пильності у мишей із позитивною якісною кореляцією між дефекацією та сечовипусканням тварин та показником грумінгу. Інгаляції ефірної олії *Litsea cubeba* призводять до зниження лише ознак вегетативної функції, а спонтанна реакція грумінгу навіть посилюється.

Висновки: Ефірна олія *Mentha piperita* зменшує середню швидкість руху тварин у центральній зоні зі збільшенням частот швидкості в периферійній зоні до вищих значень і одночасним розширенням інтервалу швидкостей; інгаляція ефірної олії *Litsea cubeba* більшою мірою активізує руху тварин у центральній зоні відкритого поля, тоді як у периферійній зоні швидкість їх рухів знижується.

Ключові слова: *Litsea cubeba*, *Mentha piperita*, психоемоційна реакція, тест відкритого поля, розподіл швидкості руху.

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Introduction. Despite the higher nervous activity and associative sphere of psychical reactions are usually inherent to humans, experiments of essential oils effects and their components on psychical action of animals is necessary and fundamental for understanding of physiological and psychological background of the aromatherapy. The comparative study of influence effects of essential oils of *Mentha piperita* L., and *Litsea cubeba* (Lour.) Pers. on behavioral reactions and psychoemotional state of mice in the open-field test was performed.

Methods: Experiments were conducted on outbred mice of both sexes, 1 min before testing exposed to inhalations of essential oils (0.05 ml of 1 % hexane solution) followed by behavioral indicators registration (squares of peripheral/central zone, time in each zone, rearing-ups, grooming, urinations, defecations etc.).

Results: Under the *Litsea cubeba* essential oil influence the most significant effect is noted on the indicator of non-supported orientation activity (increased up to 1.5 times, $p \leq 0,088$), *Mentha piperita* essential oil suppressed both this and exploratory activity reduces on 56 %. Inhalations of *Mentha piperita* essential oil induce the decrease of the level of alertness in mice with the positive qualitative correlation between defecation and urination of animals and grooming indicator. *Litsea cubeba* essential oil inhalations leads to reduction of only vegetative function signs while the spontaneous grooming reaction even increases.

Discussion: Psychological mechanisms of essential oils influence can be described by two mechanisms. The first of them is connected with associations and is based on memorizing of smells with usual imaginations. The other psychological mechanism of smells influence is subcortical, unconditionally reflex and is connected with development of olfactory unconditional reflex (stimulating, depressing, hypotensive etc.). Both *Mentha* and *Litsea* essential oils inhalations lead to changes in behavioral activity of mice in the open field test.

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Conclusion: Essential oil of *Mentha piperita* decreases the mean speed of animals movement in the central zone with the shift of speed frequencies in the peripheral zone to the higher values and simultaneous widening of speeds interval; inhalation of *Litsea cubeba* essential oil in a greater degree activates animals movements in the central zone of the open field, while in the peripheral zone their movements speed decreased.

Key words: *Litsea cubeba*, *Mentha piperita*, psychoemotional reaction, open field test, movement speed distribution.

Introduction

One of the promising areas of the medicine is the studying of new methods for actualization of organism self-organizing mechanisms and improvement of its functional abilities. The influence of essential oils is a diverse complex of pharmacological and clinical effects due to simultaneous continuous chemical evolution of smell-determining substances [1]. The smells improve the functional state of healthy system due to the increase of intersensor interactions [2, 3]. The rats with scopolamine-induced amnesia improved memory functions [4] after lavender essential oil inhalations. It had been shown that reaction time on visual stimuli shortened under influence of pleasant plant smells [5], which reflects the increase of activation level of brain neurons [6].

The olfactory feelings influence in great degree give effect on the psychical state that is why one of the main aims of aromatherapy and aromapsychology is revealing of the principles which explains and determinates essential oils effects. Despite the higher nervous activity and associative sphere of psychical reactions are usually inherent to humans, experiments of essential oils effects and their components on psychical action of animals is necessary and fundamental for understanding of physiological and psychological background of the aromatherapy. It is mainly due to that controlled and reproducible experimental animal models involve instinctive and reflex-dependent reactions, which have place in human psychics but suppressed because of socialization and not always can be determined and studied. Based on this the use of experimental animals models for obtaining the objective influence of essential oils on psychical action is the valuable scientific method for further studying of aromas and odors on humans [7, 8].

The **aim of this work** was comparative study of influence effects of essential oils of *Mentha piperita L.* and *Litsea cubeba (Lour.) Pers* on behavioral reactions and psychoemotional state of mice in the open-field test.

Materials and methods

Animals

Experiments were conducted on outbreed mice of both sexes (46 animals) which were held in accordance to the international and national bioethical recommendations on the standard laboratory diet (food and water *ad libitum* during acclimatization period) with the natural dark-light cycle [9]. Few days before experiments the animals were housed in the laboratory conditions for escaping the unnatural behavior and acclimatization. In the day of experiment animals were food deprived.

All experimental procedures conform to the guiding principles for research as recommended by "Guide for the Care and Use of Laboratory Animals" (NIH publication 86-23 revised 1985). The experiments were carried out according to the recommendations of the Committee for Research and Ethical Issues of the IASP (1983) and were approved by the regional ethical committee for animal

research of I.I. Mechnikov Odessa National University. All manipulations were made to minimize animal suffering and to reduce the number of animals used.

Inhalation chamber preparation

The inhalation chamber (glass chamber of 3 dm³ volume) was prepared for each animal individually. 10 min prior to placing the animal into the inhalation chamber the filter paper (25 cm², soaked with 0,02 cm³ 1 % (mass/volume) of essential oil solution in hexane (chosen because of chemical inertness and odorlessness) was suspended for volume saturation with vapors. The essential oil of *Litsea cubeba (Lour.) Pers* was manufactured by Beurre (UK) from fruits of *Litsea cubeba* (China) (more information is available at <https://beurre.ua/ua/essential-oil-litsea-cubeba>). The essential oil of *Mentha piperita L.* was manufactured by Aroma-Vita (India) from leaves and flowers of *Mentha piperita L.* extracted by steam distillation.

The experimental animal was placed carefully into the inhalation chamber for 1 min. After the mentioned exposition time the animal was carefully, escaping the additional stress, replaced to the open field apparatus with fixing the behavioral indicators during 3 min. After the test period the animal was placed to the common chamber.

Experimental indicators to be registered

For experiment conducting the animal was placed to the lightened apparatus near the wall (peripheral zone) and the time count started with simultaneous registration of behavioral indicators – quantity of the crossed squares of central and peripheral zones, time spent in each zone, quantity of "rearing-ups" (with or without support – near the walls), quantity of holes searching (research activity). Additionally the quantity of defecations (as boluses) and urinations as well as grooming acts was registered.

Statistical analysis of experimental data

Experimental data on each of the indicators were represented as "mean ± standard deviation from the mean in the set" (when corresponding to the normal distribution law in the Jarque-Bera test). The significance difference between data was calculated using Student t-criteria. Taking into account the animals quantity in the group and the calculated t-value the significance level for experimental group had been determined [10].

Results

The integral control indicators of behavioral activity of mice were in the range of physiological ranges and reproduced in the control indicators of two groups of animals, which have further exposed to essential oils of *Litsea cubeba (Lour.) Pers* and *Mentha piperita L.* Animals of control group most of the time spend in the peripheral zone of the "open field" chamber as it provides certain reduction of stress sense (when placed in the open space with bright light) and responds to the natural rodents behavior.

Inhalation exposition with vapors of essential oils of *Mentha piperita L.* or *Litsea cubeba (Lour.) Pers* in some degree reduce the locomotor activity (quantity of the crossed squares) in the peripheral zone (table 1) with corre-

Table 1

Influence of inhalations of essential oils of *Mentha piperita* L. and *Litsea cubeba* (Lour.) Pers on the mice locomotor activity (mean quantity of squares) in the open field test (inhalation time 1 min, test time 3 min, quantity in the group – 12 animals)

	Central		Peripheral	
	<i>Mentha piperita</i> L.	<i>Litsea cubeba</i> (Lour.) Pers.	<i>Mentha piperita</i> L.	<i>Litsea cubeba</i> (Lour.) Pers.
Control	1,91±0,08	1,68±0,09	6,1±0,58	9,32±0,95
Experiment	1,89±0,06	1,92±0,11	5,97±0,47	8,95±0,87
Significance level, p	p≤0,833	p≤0,107	p≤0,863	p≤0,775

Note: Significance level p – calculated significance level of difference

Table 2

Influence of inhalations of essential oils of *Mentha piperita* L. and *Litsea cubeba* (Lour.) Pers on the time (in absolute units (s) and per cents of all the experiment time, %) spent by animals in the central and peripheral zones in the open field test (inhalation time 1 min, test time 3 min, quantity in the group – 12 animals)

	Total time		Total time, %	
	<i>Mentha piperita</i> L.			
	Central	Peripheral	Central	Peripheral
Control	22,27±3,52	164,27±7,18	11,94±1,89	88,06±3,85
Experiment	30,25±6,11	143,58±7,15	17,4±3,52	82,6±4,11
Significance level, p	p≤0,27	p≤0,053	p≤0,185	p≤0,343
	<i>Litsea cubeba</i> (Lour.) Pers			
	Central	Peripheral	Central	Peripheral
Control	18,09±2,41	158,73±4,04	10,23±1,37	89,77±2,29
Experiment	26±6,46	168,36±6,95	13,38±3,32	86,62±3,58
Significance level, p	p≤0,263	p≤0,244	p≤0,39	p≤0,467

Note: Significance level p – calculated significance level of difference

sponding increase of it in the central zone, though without statistically significant difference.

According to the obtained data there is a redistribution between animals locomotor activity in central and peripheral zones which can be explained as partial reduction of stress state and mild anxiolytic effect after exposition to essential oil vapors. It has also be mentioned that despite this effect is not statistically significant (the calculated significance level p is not lower than standard 0,05) the *Litsea cubeba* (Lour.) Pers. essential oil effect is more considerable in compare to that of *Mentha piperita* L. (table 1).

The time spent in each of these zones more closely correlates with the stress level and anxiolytic effect as it is the direct result of animals exposition to stresogenic conditions (staying either on the open space, or near the walls of the chamber). And for this indicator essential oil of *Mentha piperita* L., which was chosen as reference substance, exhibits the effect which is close to statistically significant for the absolute values for peripheral zone p≤0,053). The redistribution of the total time spent in the central and peripheral zones is more marked when transforming the values to per cent values and it is noted that (table 2) while essential oil of *Mentha piperita* L. increases the relative time of stress zone (central) staying (up to 1,5 times), essential oil of *Litsea cubeba* (Lour.) Pers also demonstrates the slight similar effect (table 2).

Under the influence of essential oil of *Mentha piperita* L. there is noted decrease (not statistically significant) of “rearing-ups” number both supported and unsupported (p≤0,897 and p≤0,642 correspondingly). The more prom-

inent is the influence of essential oil of *Mentha piperita* L. on research activity which is statistically significantly (p≤0,031) decreased up to 56 %. Though the same indicator for *Litsea cubeba* (Lour.) Pers has no statistically significant difference (p≤0,088) the free research activity of animals increased to 1.5 times. Because this type of activity, as had been mentioned, is combined with higher stress for animals, one can assume that inhalations of *Litsea cubeba* (Lour.) Pers essential oil lead to calming effect on experimental animals.

Mentha piperita L. essential oil inhalation caused the decrease of the mentioned behavioral indicators of animal’s psychoemotional state (table 3). Though none of the indicators showed the statistically significant difference between control and experimental groups, there is a tendency or mice anxiety reduction with the positive qualitative correlation between defecation, urination and grooming. On the contrary, *Litsea cubeba* (Lour.) Pers essential oil inhalations leads to reduction of only vegetative function signs while the spontaneous grooming reaction even increases (table 3). Such increase, combined with reduction of emotional stress can be regarded as external manifestations of adaptive reaction as well as stress state mitigating, thus even without statistically significant difference between control and experimental group, one can assume that *Litsea cubeba* (Lour.) Pers. essential oil possess calming (anxiolytic) effect.

Because in the experiment there were determined both the quantity of the crossed squares and the time of their crossing (or being there), it had also been estimated the

Influence of essential oils of *Mentha piperita* L. and *Litsea cubeba* (Lour.) Pers on the psychoemotional activity and spontaneous reactions of mice in the open field test (inhalation time 1 min, test time 3 min, quantity in the group – 12 animals)

	Psychoemotional activity		
	Defecations	urinations	grooming
	<i>Mentha piperita</i> L.		
Control	2±0.41	4±0	4±2.02
Experiment	1.5±0,5	1±0	2.56±0.34
Significance level, p	p≤0.447	-/-	p≤0.488
	<i>Litsea cubeba</i> (Lour.) Pers		
Control	2±3.25	1±1	1±2
Experiment	1.5±0.5	1±0	2.43±0.72
Significance level, p	p≤0.881	-/-	p≤0.508

Note: Significance level p – calculated significance level of difference “defecations” – quantity of boluses, “urinations” – quantity of urinations, “grooming” – quantity of grooming acts, «-/-» – the value is not determined.

mean speed of mice movements in the open field before and after essential oils inhalations. The more informative is the frequencies distribution (in % of the total quantity) of movement speed of animals in the separate zones of the open field (Fig. 1-2).

Attracts attention the fact of trimodal distribution of animals speed in the central zone of the open field (fig. 1A, 2A) – the animals demonstrate clearly identifiable speeds of movement from the lowest one (about 0.6 squares/s), which can be directly attributed to the locomotor activity in the “orientation” state and field researching, to the highest one (to 2 squares/s) which can be a sign of anxiety state and emotional tension. There is also stands out the movement frequency with 1.0-1.2 squares/s, which is attributed to the motion of the animal between the explored squares.

On the contrary, in the peripheral zone, where the fear sense of animals was reduced, the distribution of frequencies of movement speeds has monomodal (unimodal) profile with maximal speed of 0.4-0.6 squares/s. This type of movements can be attributed to the slow exploration of the environment in the conditions of reduced stress.

Inhalation of essential oils also induces the certain redistribution in the frequencies of animals speed and the effects of *Mentha piperita* L. and *Litsea cubeba* (Lour.) Pers are different. So, the inhalation of *Mentha piperita* L. essential oil reduces the mean speed of animals movements in the central zone (Fig. 1A) with the certain shift of the speed frequencies in the peripheral zone to the higher values and concomitant widening of the speeds interval (Fig. 1B). The reducing of the feeling of stress and fear stimulate the animals to more freely explore the environment, because of which becomes wider the dispersion of speeds due to the individual differences in the psychical activity.

Inhalation of *Litsea cubeba* (Lour.) Pers essential oil in a greater degree activates the animals movements in the central zone of the open field (Fig. 2A), while their movements speed in the peripheral zone reduces (Fig. 2B).

Discussion

Psychological mechanisms of essential oils influence can be described by two mechanisms. The first of them is connected with associations and is based on memorizing of smells with usual imaginations. For example, stimulating smells (claw, pepper, iris, coffee) arose warm,

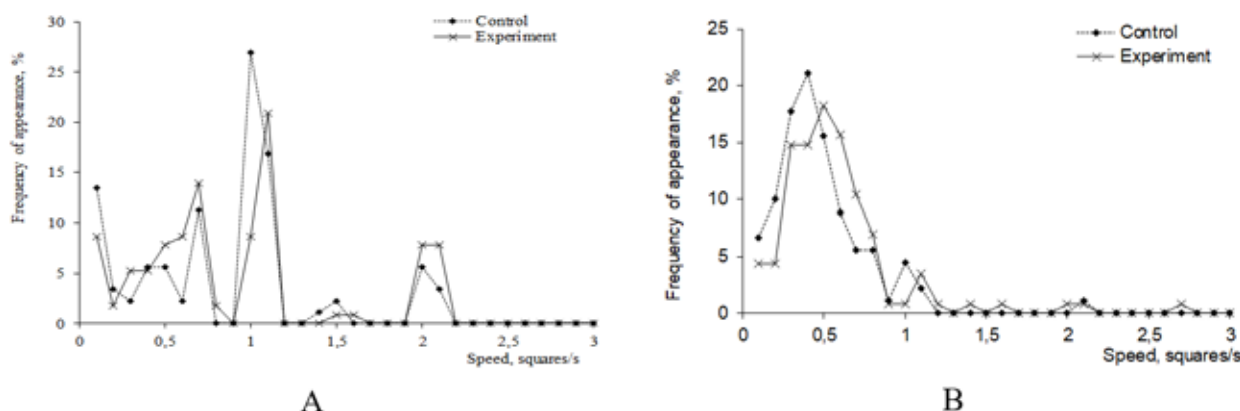


Fig. 1. Influence of *Mentha piperita* L. essential oil on the locomotor activity (relative frequency of movements speed, squares/s) of mice in the central zone (A) and in the peripheral zone (B) of the open field test (inhalation time 1 min, test time 3 min, quantity in the group – 12 animals)

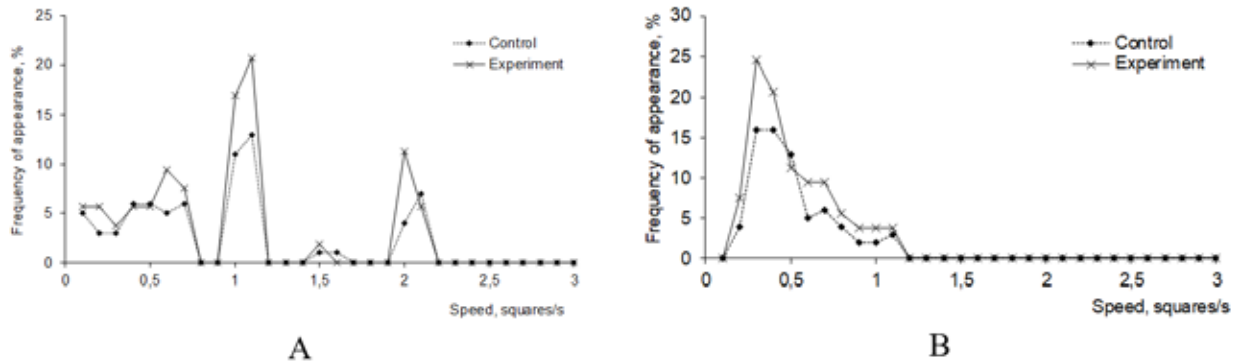


Fig. 2. Influence of *Litsea cubeba* (Lour.) Pers essential oil on the locomotor activity (relative frequency of movements speed, squares/s) of mice in the central zone (A) and in the peripheral zone (B) of the open field test (inhalation time 1 min, test time 3 min, quantity in the group – 12 animals)

pleasant memories, thus stimulating the nervous system. The other psychological mechanism of smells influence is subcortical, unconditionally reflex and is connected with development of olfactory unconditional reflex (stimulating, depressing, hypotensive etc.). There is an opinion that in this case the smell carrier have to be complementary to the corresponding receptor [11, 12, 13] but, taking into account the diverse systems and interactions of different departments of the nervous system and cognitive functions involved [14, 15], the unconditionally reflex mechanism can be one of the most real in essential oils effects realization.

The essential oil of *Litsea cubeba* (Lour.) Pers was obtained only the last century due to which the practical experience of its use is not so deep as for other traditional essential oils. But the main tendency in its use is based on the *Litsea cubeba* (Lour.) Pers essential oil normalization of the cardiovascular system, heart arrhythmia regulating, reducing of high blood pressure, improving the bronchospastic syndromes, calming down the neurotic headache as well as effectiveness for other kinds of pain (for example, muscular). There is the information that *Litsea cubeba* (Lour.) Pers essential oil reduces excessive excitement, decreases depressive and astenoid symptoms and counteracts to insomnia. Thus *Litsea cubeba* (Lour.) Pers essential oil can be regarded as sedative remedy with potential to normalize the psychical processes. Paying attention to the abovementioned it had been estimated the influence of *Litsea cubeba* (Lour.) Pers essential oil inhalation on psychoemotional state and some indicators of behavioral reactions of experimental animals (mice). As reference there was chose the essential oil of *Mentha piperita* L. which share well-known sedative action.

Both *Mentha* and *Litsea* essential oils inhalations lead to changes in behavioral activity of mice in the open field test.

The observations of such differences can be due to the fact that in the stressogenic conditions (central zone of the open field chamber) the animals are more intensively explore the environment because of movements and are in the more active state. In the peripheral zone of the open field, where the stressogenic tension is expressed in less degree, the animals are more quietly and slowly explore

the environment and move, correspondingly more slowly. But even in this case there is a “widening” of the animals speed frequencies interval, as it had been noted for effect of *Mentha piperita* L. essential oil inhalation, which is due to the greater manifestation of individual differences of animals.

In general, one can summarize that though the influence of the *Mentha piperita* L. essential oil on the behavioral reactions of the experimental animals is not strong and for most of indicators has no statistically significant difference, there can be distinguished some effects directions, which manifest in motion activity and emotional tension reduction. The effect of essential oil of *Litsea cubeba* (Lour.) Pers mostly appears in adaptive changes of the behavioral reactions in the stressogenic conditions (central zone), suppression of movement activity in the peripheral zone and mild calming (anxiolytic) action, which manifests in reduction of vegetative reactions (defecation and urination) together with increased feeling of comfort (increased grooming acts).

The distribution of movement speed in the central zone of the open field chamber for control animals is trimodal and in the peripheral zone is unimodal. Inhalation of essential oils also induces the redistribution in the frequencies of animals speed and the effects of *Mentha piperita* L. and *Litsea cubeba* (Lour.) Pers are different. Essential oil of *Mentha piperita* L. decreases the mean speed of animals movement in the central zone with the shift of speed frequencies in the peripheral zone to the higher values and simultaneous widening of speeds interval; inhalation of *Litsea cubeba* (Lour.) Pers. essential oil in a greater degree activates animals movements in the central zone of the open field, while in the peripheral zone their movements speed decreased.

Conclusions

After the inhalations of *Mentha piperita* L. essential oil there is noted the substantial suppression of orientation activity of animals (“rearing-ups”) and the exploratory activity reduces on 56 % due to the calming effect. Under the *Litsea cubeba* (Lour.) Pers essential oil influence the most significant effect is noted on the indicator of non-supported orientation activity (increased up to 1.5 times, $p \leq 0.088$).

Inhalations of *Mentha piperita* L. essential oil induce the decrease of indicators of psychoemotional state (the level of alertness in mice) with the positive qualitative correlation between defecation and urination of animals and grooming indicator. On the contrary, *Litsea cubeba* (Lour.) Pers essential

oil inhalations leads to reduction of only vegetative function signs while the spontaneous grooming reaction even increases.

In general, *Litsea* essential oil inhalation induces different changes on behavioral activity of mice in stressogenic conditions which have to be further detailed.

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