

In Solitary Rabbits, Does the Presence or Absence of a Mirror Affect Stress, Fear and Anxiety?

A Knowledge Summary by

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KNOWLEDGE SUMMARY

PICO question

In solitary rabbits, does the presence or absence of a mirror affect stress, fear and anxiety?

Clinical bottom line

Four studies were reviewed in this knowledge summary. Despite the small number of papers available, the controlled trials and their methodologies provide strong evidence that the behavior of solitary laboratory rabbits is affected by the presence or absence of mirrors. This evidence also indicates an effect on their welfare.

The studies suggest that the presence of mirrors provides environmental enrichment and have a positive effect on the subjects health and welfare. However, it is also suggested that mirrors may have some detrimental physical and psychological effects for some individuals. There are also many gaps in the available research and these need to be addressed to give better insight into the effect of mirrors on rabbits.

Because of the current level of knowledge, clinical recommendations cannot be made at present on the use of mirrors in solitary rabbits to improve their veterinary care whilst hospitalised.

Clinical Scenario

Rabbits are social animals and require same species companionship in captivity. From recent rabbit CPD courses run by LagoLearn and The Rabbit Welfare Association & Fund, the accepted gold standard of rabbit veterinary care is to also bring bonded companions into practice during periods of hospitalisation. Mirrors have been successfully used and proven to reduce stress in other isolated social species; sheep (Parrott et al., 1988); cattle (Piller et al., 1999); horses (McAfee et al., 2002). Could mirrors help reduce stress in rabbits? Could the use of mirrors be advantageous for solitary housed rabbits in a veterinary practice, subsequently improving their recovery time, nursing care and welfare?

The evidence

The literature searches found four relevant papers (Jones & Phillips, 2005; Dalle Zotte et al., 2008; Edgar & Seaman, 2010; Reddi et al., 2011) that directly compared whether presence or absence of mirrors influenced rabbit behaviour. None of these directly measured stress, fear or anxiety, however all assessed preference for mirrors by observing rabbit behaviour instead. The quality of evidence was strong due to the use of controlled trials in all research papers. All studies suggested that in laboratory rabbits, mirrors had some welfare advantages. No evidence was found on the use of mirrors in a domesticated or veterinary setting.



next review date: 12 April 2021

Summary of the evidence

Population:	Weaned New Zealand White rabbits, weighing 450–500 g on a
r opulation.	private rabbit farm in Bangalore, India
Sample size:	10 rabbits split into two treatment groups:
	$-T_1(n=5)$
	- T ₂ (n = 5)
Intervention details:	Experimental set up
	 T₁ rabbits were housed individually in cages without a mirror T₂ rabbits were individually housed in cages with a mirror on one wall only
	<u>Further experimental detail</u>
	 All rabbits were provided with feeders and drinking cups
	- Rabbits had access to ad libitum green grasses and
	concentrate feed each day, fed at 0900h and 1400h
	respectively - Temperature was controlled at 24°C
	- Lighting was controlled, rabbits had 16 hours of light per day
	Methodology
	- Rabbit bodyweights were recorded weekly on electronic
	weighing scales
	- Rabbits were observed for their behavioural activities. It is
	not clear from the research methodology how this outcome was measured
	- Data was analysed using a t-test (Snedecor & Cochran, 1968)
	Data was analysed asing a cress (shedeson a soomany 1995)
	Experimental timeline
	- Study duration was 6 weeks
Study design:	Controlled trial
Outcome studied:	The influence of presence or absence of a mirror on:
	 Growth performance including average daily and weekly
	bodyweight gains
	- Behaviour changes including; activities, performance,
	feeding preferences, comfort and other notable behaviours
	ex. scrabbling at the mirror
Main findings: (relevant to PICO question):	Growth performance - T_1 rabbits (without mirror) weighed 459 g and 844.2 g on
(relevant to FICO question):	average in the first and sixth week respectively
	 Mean weight for rabbits in T₁ over the 6 week trial period
	was 638.07 g (P<0.05)
	- T ₂ rabbits (with mirror) weighed 436 g and 1017 g on
	average in the first and sixth week respectively



	was 729.87 g (P<0.05) - Rabbits individually housed with a mirror in their cage had higher average weight gains throughout the trial period
	Behaviour changes - T ₂ rabbits were more active - T ₂ rabbits tried to look at the mirror most of the time, including when feeding - T ₂ rabbits consumed all feed without residues - T ₂ rabbits preferred the cage side and to remain in comfortable positions
Limitations:	 Small sample size Only one breed of rabbit used Increase in bodyweight may not mean better welfare for the rabbits. A barren experimental environment could mean that feeding is the only enrichment and stimulation received each day, so rabbits may have spent more time eating in general as that was the only activity available Frequency of behaviour (activity, looking into the mirror whilst eating) for T₂ rabbits housed individually with a mirror remained significant until decreasing after 2 weeks into the trial. This could be due to habituation to the mirror as the rabbit did not receive confirmatory cues that the image was a conspecific Limited insight into methodology for behavioural measurement and assessment so unable to gain insight into how the data was acquired

Jones & Phillips (2005)	
Population:	Unrelated female rabbits aged between 7 and 12 months obtained from a breeder
Sample size:	Six rabbits of two breeds: - Dwarf Lop (n = 3) - Lionhead (n = 3) Four rabbits were split into four treatment groups (two of each breed): - T ₁ Isolated without mirrors (n = 1) - T ₂ Partially isolated without mirrors (n = 1) - T ₃ Isolated with mirrors (n = 1) - T ₄ Partially isolated with mirrors (n = 1) - The other two rabbits were used as conspecific stimuli and were not observed
Intervention details:	 Experimental set up All test rabbits had access to a cage with two sections, separated by a small door Section 1 was approximately 1700 cm² in size and had four solid sides, including a small door into section two



- Section 2 in isolated rabbit treatment groups (T_1 and T_3) was 2400 cm² with three solid sides and a wire mesh grill at the front

- Section 2 in partially isolated rabbit treatment groups (T₂ and T₄) was the same size, had two solid sides, a wire mesh grill at the front but could also see another rabbit through a plastic mesh panel attached to a single cage. These rabbits will not be discussed in this knowledge summary as this is not relevant to the PICO
- Mirrors were attached to the walls of Section 2 and did not run parallel to each other to avoid multiple reflections

Further experimental detail

- Cage floors were covered in newspaper and straw
- Rabbits had access to ad libitum hay and water provided in drinking bottles
- Rabbits were offered a daily carrot at 0900h
- Fresh hay and 30 g concentrate were offered at 1600h
- Cages were weather proofed with roofing felt
- Cages were outside but positioned facing a building for shelter ensuring rain could not enter

Methodology

- Initial observations were done 1h after rabbits were placed in their test environment on day 1 at 1500h
- On day 7, rabbits were observed from 0800h to 1430h
- The duration and number of occurrences of all behaviours were recorded at the end of every 1 minute period
- Behaviour was divided into two categories via a post hoc analysis; 'long duration' (lasted for more than a minute on more than 50% of occasions) and 'short duration' (all other behaviour)
- Rabbits were randomly allocated to treatment groups using a balanced Latin Square (Davis and Hall, 1969)

Experimental timeline

- Study duration was 1 week

	- Study duration was 1 week
Study design:	Randomised controlled trial
Outcome studied:	The effects of provision or absence of mirrors on behaviour duration
	and frequency of behaviour exhibition over time. The time periods
	observed were:
	- Initial response
	- Response over time (comparison between day 1 and day 7)
Main findings:	<u>Initial response</u>
(relevant to PICO question):	 Rabbits housed with a mirror showed some alert behaviour, whereas rabbits without a mirror did not

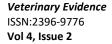


	 Mirror presence doubled sniffing behaviour initially Mirror presence did not affect sniffing of the wire mesh grill at the front of the cage More exploratory behaviour performed initially
	Response over time (comparison between day 1 and day 7) - Mirror presence had a stronger effect in increasing sniffing of vertical surfaces than those without - Decreased the time rabbits spent looking out of their cage - Increased the time rabbits spent sitting - Increased the frequency of multiple behaviours observed over every 1 minute period
Limitations:	 Females only so the results cannot be generalised to include all rabbits as males may have reacted differently A small sample size was used and there was only one rabbit in each treatment group It was not stated in the study how the observations were carried out

Edgar & Seaman (2010)	
Population:	New Zealand White rabbits aged between 15–16 weeks old
Sample size:	24 rabbits, both males and females who had been singly housed from weaning: - Males (n = 9) - Females (n = 15)
Intervention details:	 Experimental set up Rabbits were individually housed in standard metal laboratory cages in the top two rows of a standard three row rack of cages Positioned facing a wall (1.5 m away)
	 Further experimental design Test rabbits were not in visual contact but had olfactory and auditory contact as all rabbits were housed in the same room Ad libitum access to standard rabbit pellets and water Controlled lighting; light hours were between 0530h and 2330h Urine and faeces were removed twice weekly Rabbits were also used for antibody production with blood being collected every week. They were not handled at any other time
	Methodology - Rabbits were split into three groups of 8 cages for filming. Rabbits were continuously recorded using two CCTV cameras



to film four cages each Observations were split into three sections: 1. **Pre-trial observations** (day 1–3) Each rabbit acted as its own control to provide a baseline behavioural assessment. 2. Mirror trial observations (day 4–10) An acrylic mirror was attached to the inside of the cage at either the front (males n = 4; females n = 8) or back (males n = 8) = 5; females n = 7). This was randomly assigned on the left or right of the cage. 3. Post-trial observations (day 11–13) Mirrors were removed and behaviour observed and recorded for a further three days. Observation schedules and behavioural assessment was validated using a pilot study (2 day pre-trial, 13 day mirror trial, 7 day post-trial) Experimental timeline Study duration was 13 days Study design: Randomised controlled trial **Outcome studied:** Gender Percentage of time rabbits spent performing behaviours in each behavioural category; inactive, eating and drinking, body maintenance, mobility, stereotypy, investigatory behaviour, comfort behaviour, mirror related behaviour, time spent at the front and back of the cage Main findings: <u>Gender</u> (relevant to PICO question): Female rabbits spent less time on body maintenance than males. There was a significant interaction between period and gender (P = 0.006) with females and body maintenance, showing a significant reduction in body maintenance at the end of the trial compared to at the start Male and female rabbits spent on average 1.2% and 0.8% of time, respectively, engaged in stereotypic behaviour ex. bar biting and scratching at the floor of the cage Females showed a more consistent level of stereotypic behaviour ex. bar biting and scratching at the floor of the cage Males showed an initial increase in stereotypic behaviour in the pre-trial period. This decreased during the mirror period and subsequently increased again in the post-trial period (P = 0.042) Males exhibited more investigatory behaviour than females, although this was similar in pattern across both genders. Investigatory behaviour increased during day 1–2 and then subsequently declined over time Females spent 0.21% of time sniffing, scratching and biting the mirror





	 Males spent 0.15% of time sniffing, scratching and biting the mirror
	 Females spent more time at the front of the cage in the
	mirror trial period [31%] than in the pre-trial period [25%] (P
	= 0.017)
	- 0.017)
	Behaviour performance
	- All rabbits showed an increase in investigatory behaviour (P
	= 0.042)
	- All rabbits showed an increase in inactive behaviour over
	time during the mirror-trial period (P = 0.014)
	time daring the mirror that period (1 = 0.014)
Limitations:	 Investigatory behaviour declined over time. This could be
	due to habituation to the mirror and the rabbit not receiving
	confirmatory cues that the mirror image was another rabbit
	- Could not distinguish between grooming (body
	maintenance) and hair chewing (stress response/coping
	mechanism)
	- Different social strategies between males and females could
	have affected the results. For example, males showed
	• •
	increased investigatory behaviour at the start which was
	more so than females. This may have contributed to stress if
	the mirror image was perceived as a conspecific, and
	therefore did not positively influence male rabbit welfare
	 It was not clear in the mirror trial period of the study if the
	mirror positioning at either the front or back of the cage had
	an effect on the rabbits behaviour

Dalle Zotte et al. (2008)	
Population:	Pannon White rabbits aged 5 weeks old [sic], housed in a closed, climatised rabbitry located in Kaposvár University, Hungary
Sample size:	 56 [sic] rabbits split into two treatment groups: Individually housed (n = 18) Group housed (n = 2, 28 rabbits in total, 1 group of 12 and 1 group of 16)
Intervention details:	 Experimental set up Individual cages had a basic area of 0.24 m² Individual cages had a stocking density of four cages per m² with one rabbit in each cage Two group pens had an area of 1 m² each Both group pens and individual cages were split into two parts; one side was covered in mirrors and one side was covered in plastic panels Each side of all pens were separated by a swinging door
	Further experimental detail - Temperature was controlled at 18°C - Ad libitum commercial pellet diet and water provided



	- Controlled lighting; 16h light and 8h dark per day
	 Methodology Each treatment group was used as its own control Random assignment of rabbits to pens 24h video recording performed twice weekly using infrared cameras Rabbits were not disturbed by human presence on the days of recording The number of rabbits in each pen was counted every 15 minutes, a total of 96 times per day Rabbit preference was assessed using a chi-squared test
	Experimental timeline - Study duration was 6 weeks The study assessed stocking density, age and the effect of the time
	of day on rabbits' preference for cages with or without a mirror. Only data from individually housed rabbits will be used in this knowledge summary as this is the only data relevant to the PICO.
Study design:	Randomised controlled trial
Outcome studied:	Percentage of time spent in cages with mirrors including: - By age - By time (active [dark] vs. inactive [light] periods) - Overall (throughout the 6 week study duration)
Main findings: (relevant to PICO question):	By age - 74.2% of rabbits aged 5.5 weeks [sic] preferred the mirrored part of the cage (p<0.001) - 67% of rabbits aged 11.5 weeks preferred the mirrored part of the cage (p<0.001) - Rabbits were attracted to their mirror reflection up to 11 weeks old - Mirror preference decreased slightly with age
	By time period - During the active (dark) period, 70.9% (p<0.001) of rabbits were found to prefer the mirrored part of the cage - During the inactive (light) period, 70.1% (p<0.001) of rabbits preferred the mirrored part of the cage
	Overall - 67% of individually housed rabbits showed a preference for the part of the cage enriched with mirrors (p<0.001)
Limitations:	 Preference for mirrors decreased as the rabbits got older. However, this could be due to habituation to mirrors and becoming more familiar with them in the environment. They did not receive confirmatory cues that the mirror image was



- a conspecific so could have reduced interest
- Sample was restricted as all rabbits were between 5 [sic] and 11.5 weeks old. This could be potentially useful for the hospitalisation of young rabbits, but would not be generalisable to all rabbits as there was no indication of mirror preference in older rabbits
- 6 weeks in duration which is a longer amount of time than a rabbit would generally be hospitalised for. Although there may be some cases requiring longer hospitalisation depending on the aetiology
- Minimal enrichment in cages, which would be similar to that
 of veterinary hospitalisation. Did rabbits prefer the mirror
 because it was the only thing to interact with in the
 environment? Was this down to curiosity instead of
 recognising the mirror image as a conspecific?
- The study involved pens which would be familiar to laboratory rabbits but not to pet rabbits. It is an unnatural, artificial environment and may therefore not be generalisable wholly to domesticated rabbits
- The information in this Knowledge Summary is taken directly from the paper, it is unclear whether the rabbits were aged 5 or 5.5 weeks at the start of the study and 11 or 11.5 weeks at the end respectively

Appraisal, application and reflection

All four studies addressed the effects of introducing a mirror to a solitary rabbits environment. It is important to note however, that the experimental timeline of all studies was generally longer than the average rabbit would spend hospitalised in veterinary practice.

Stress, fear and anxiety, as outlined in the PICO, were not directly measured although all four studies concluded that the presence of mirrors did in fact affect the subject rabbits behaviour. All studies measured behavioural responses, some of which could be indicative of a stressed, fearful or anxious rabbit.

As an example, fearful or anxious behaviour could include, but is not limited to, banging hind feet, darting and tachypnoea. Also, expression of stereotypies such as hair chewing has been suggested by previous studies to be indicative of stress due to social deprivation (Gunn & Morton, 1995). Therefore, if a rabbit recognises a reflective mirror image of itself as another rabbit, this psychologically provides companionship. This was the case in female rabbits in Edgar & Seaman (2010) as a significant interaction was observed. Female rabbits spent less time on body maintenance during the mirror trial. So, if addition of a mirror reduces social deprivation through provision of a conspecific image, this may lead to reduced exhibition of stress-related behaviour. Combined, this can improve rabbit welfare for short term periods of hospitalisation in a veterinary practice.

However, it is important to note that no differentiation between grooming (normal behaviour, generally considered positive) or hair chewing (stress-related behaviour) could be made during this study. Body maintenance was previously considered to be at elevated levels as each rabbit was used as its own control. The quality of this research was good due to it being a controlled trial, however, it was unclear from the methodology as to what constituted as a 'high level' of body maintenance prior to commencing behavioural observations. Regardless, body maintenance did significantly decrease after introduction of the mirror. This requires further investigation to be able to differentiate between normal and stress-related behaviour, grooming and hair chewing respectively. If both were previously at higher levels than normal, this could be



indicative of stress anyway. Therefore, in relation to the PICO, presence of a mirror could reduce stress-induced over-grooming in female laboratory rabbits although further research is needed with a larger sample before this result can be applied to improving welfare and nursing care of hospitalised rabbits.

Taking this into consideration, both the variety of behaviour expressed and activity levels of rabbits housed with mirrors decreased over time also. Jones & Phillips (2005) found rabbits were initially attracted to mirrors placed in their cages and spent time scrabbling at it, although this behaviour was observed less often over time. This study was very limited however as breed and treatment were confounded. Activity of rabbits also gradually decreased throughout the duration of the study in Reddi et al. (2011). It was suggested in both studies that this was because test rabbits did not receive confirmatory cues that the mirror was a conspecific and became less interested over time. They then became habituated to the mirror. It could therefore be suggested that, short term, a mirror could be beneficial and provide welfare advantages through environmental enrichment.

Similarly, mirror preference was suggested to decrease as the rabbits got older in Dalle Zotte et al. (2008). It was shown that rabbits were attracted to the mirror up to 11 weeks of age and the strongest preference was at the start of the study when the test rabbits were 5.5 [sic] weeks old. It is therefore unclear if the contributing factor towards mirror preference was the age of the rabbits or time spent with the mirror. In this paper, the ages of the rabbits do not add up, however the data was taken directly from the published study. In relation to the PICO, it is likely that younger rabbits show a stronger preference towards mirrors although all rabbits may be initially interested in them if introduced to their immediate environment. Further research could control rabbit age and offer a better insight into this finding.

On the other hand, Edgar & Seaman (2010) contradicts the idea of habituation. Body maintenance continued to decrease over time. If this was stress-related behaviour, this strengthens the argument that mirrors have welfare advantages for rabbits, particularly those hospitalised short-term. However it was unclear from this study whether the position of the mirror within the cage also had an effect on rabbit behaviour. The fact stress-related body maintenance decreased significantly overall also suggests female test rabbits recognised the mirror image as a conspecific, subsequently improving welfare by psychological provision of companionship.

Another behaviour that decreased over time was investigatory behaviour, although some aspects of this may not be advantageous to welfare. For example, many of the studies above (Jones & Phillips, 2005; Edgar & Seaman, 2010; Reddi et al., 2011) stated in the initial experimental periods that investigatory behaviour increased, including sniffing and scratching at not only the mirror but vertical surfaces. In relation to the PICO, this strengthens the idea that a mirror could be considered a good and relatively cheap form of environmental enrichment in a veterinary setting for short term hospitalisation. However, before any clinical recommendations can be made, further research is needed into specific factors affecting this, particularly the initial response of male rabbits to mirrors.

It was suggested mirrors could be detrimental to male rabbits as vigilance and alertness increased initially, and more dramatically than females when introduced to a mirror (Edgar & Seaman, 2010). The reasoning for this is not clear, however it is not unreasonable to suggest this could be due to competition for resources and territory with the mirrored image, especially being in a small and confined space. It is important to assess whether increased alertness also leads to increased levels of stress, fear and anxiety. It is important to investigate this before being able to recommend mirrors as nursing consideration for hospitalised male rabbits. Social factors behind these results need to be identified and assessed to avoid negative effects on welfare.

As well as reducing some behaviour, mirror presence has shown to be advantageous in improving some aspects of rabbit husbandry such as food consumption. Reddi et al. (2011) found the presence of mirrors increased rabbit bodyweight, food consumption and feeding efficiency. This was also found in Jones & Phillips

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(2005) although hay intake was lower initially in this study. In relation to the PICO, it is important to consider these results could potentially be detrimental to rabbit welfare and increase stress, fear and anxiety. The presence of a mirror could increase food intake due to competition and resource rivalry with conspecific images. However, this does assume that rabbits are able to distinguish the mirror image from their own reflection and recognise it as another rabbit. It is assumed in all studies outlined above that rabbits are not capable of self-recognition. In a veterinary setting, mirrors could potentially aid inappetence in rabbits however further investigation into influencing factors, particularly in male rabbits, is needed before this can be recommended.

Considering all the above in relation to the PICO, there have been some benefits to rabbit welfare through using a mirror as environmental enrichment when assessed using behavioural observations. However, there have also been some indications that mirrors may be detrimental to the welfare of some rabbits. It is important to note that rabbits in a hospitalised environment will most likely be unwell or injured. They may prefer to be alone opposed to being housed with an unfamiliar conspecific image. This highlights the need for further, well controlled research studying both healthy and unwell rabbits, as well as a variety of treatments. There is also a need for larger sample sizes in future studies as this would add more confidence to any results before any practical application can be taken from existing research.

In conclusion, solitary rabbits are affected by the presence or absence of a mirror in their environment, but it is difficult to make definitive recommendations as there are large knowledge gaps currently in available published research.

Methodology Section

Databases searched and dates	A. PubMed via the NCBI website (01/01/1973 – 31/12/2017
covered:	B. CAB Abstracts on the OVID interface (1973 – 2017 Week
covered:	B. CAB Abstracts on the OVID Interface (1973 – 2017 Week
Search terms:	<u>PubMed</u>
	1. (rabbit OR rabbits OR lagomorph OR lagomorphs OR
	"oryctolagus cunniculus" OR bunny OR bunnies OR hare C
	hares OR buck OR bucks OR doe OR does OR kittens OR ki
	OR kitten) AND
	2. (mirror OR mirrors OR "reflecting glass") AND
	3. (stress OR stressed OR anxiety OR fear OR anxious OR fea
	OR worried OR nervous OR nervy OR panic OR panicked C
	fright OR frightened OR "tonic immobility" OR ti OR
	immobility OR distressed OR distress OR scared OR scary
	afraid OR behavioural OR pressure OR tension OR alarm C
	alarmed OR vigilant OR vigilance OR apprehension OR
	apprehensive OR activity OR time OR budget OR "time
	budget" OR nervousness OR stressful OR distress OR pain
	painful OR "pain score" OR "pain scoring")
	CAR Abstracts
	CAB Abstracts
	 (rabbit or rabbits or lapine or lagomorph or lagomorphs bunny or bunnies or hare or hares or buck or bucks or de
	or kittens or kits or kittens or oryctolagus).mp. or exp
	rabbits/ or exp oryctolagus cuniculus/
	2. (mirror or mirrors or reflector or reflective or reflection
	'reflecting glass' or 'reflective glass')
	3. (stress or stressed or stressful or distressed or anxiety or





	 anxious or fear or fearful or fearfulness or worried or nervous or nervy or nervousness or panic or panicked or fright or frightened or scared or scary or afraid or alarmed or alarm or apprehension or apprehensive or behaviour or behaviour).mp. or exp stress/ or exp fearfulness/ or exp anxiety/ 4. 1 and 2 and 3
Dates searches performed:	A. 05/12/2017 B. 16/10/2017

Exclusion / Inclusion Criteria

Papers were screened by titles and abstracts initially upon whether they met the criteria to answer the knowledge summary question. Upon meeting the inclusion criteria, the full text article was obtained and assessed

assessed	
Exclusion:	 Irrelevant to PICO question Paper subjects were not rabbits No use of mirrors Non-English publications Duplicates
Inclusion:	 Primary research papers Peer reviewed journals Rabbit subjects Comparison of presence or absence of mirrors Relevant to PICO question Publications in English language

Search Outcome				
Database	Number of results	Excluded – not relevant to PICO	Excluded – duplicates	Total relevant papers
PubMed	106	106	0	0
CAB Abstracts	12	7	1*	4
Total relevant papers when duplicates removed				4

^{*}Paper was first presented at Proceedings of the 9th World Rabbit Congress, Verona, Italy, 10–13 June 2008

CONFLICT OF INTEREST

The author declares no conflict of interest.



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