ISSN: 2038-3282

Pubblicato il: gennaio 2024

Registrazione Tribunale di Frosinone N. 564/09 VG

Olfactory LearNap: olfactory stimulation during learning and nap to strengthen memorization in early childhood

Olfactory LearNap: stimolazione olfattiva durante l'apprendimento e il sonno pomeridiano per rafforzare la memorizzazione nella prima infanzia

di

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Abstract:
This pilot study investigates whether olfactory stimulation, proposed during both learning and naps for children aged 2-3 years, can impact the consolidation of a mnemonic trace. Research demonstrates howsleep plays a central role in this process, and, in early childhood, promotes brain development. The senseof smell, active during sleep, supports learning and memory, due to the closely connections of olfactorybulb to the hippocampus and amygdala. It is quantitative experimental research that compares the acquisition of specific information before and after a language training protocol in two groups. The experimental group, who benefited from olfactory stimulation during both the learning

and sleep phases,have revealed an average improvement of 23%, due to the hippocampal reactivation of the learned content facilitated by olfactory stimulation. This research demonstrates that intervention with olfactory stimuli can indeed lead to improvements in memorization in early childhood.

Keywords: Kindergarten; olfactory memory; sleep; memory consolidation; children development.

Abstract:
Questa ricerca pilota indaga se la stimolazione olfattiva, proposta sia durante l'apprendimento che il riposo pomeridiano, per bambini di 2 e 3 anni, possa incidere sulla consolidazione di una traccia mnemonica. Il sonno gioca un ruolo centrale in questo processo e, nell'infanzia, favorisce lo sviluppo cerebrale. Il senso dell'olfatto, attivo durante il sonno, supporta l'apprendimento e la memoria grazie allestrette connessioni del bulbo olfattivo con l'ippocampo e l'amigdala. È una ricerca sperimentale quantitativa che confronta, in due gruppi, l'acquisizione di informazioni prima e dopo un protocollo linguistico. Il gruppo sperimentale, che ha beneficiato della stimolazione olfattiva durante la fase di apprendimento e di sonno, ha mostrato un miglioramento medio del 23%, dovuto alla riattivazione ippocampale dei contenuti appresi facilitata dalla stimolazione olfattiva. Questa ricerca dimostra che l'intervento con stimoli olfattivi può portare a miglioramenti nella memorizzazione nell'infanzia.

Parole chiave: asilo nido; memoria olfattiva; sonno; consolidamento mnestico; neurosviluppo.

1. Introduction[1]

"We are who we are because of what we learn and what we remember" (Kandel et al., 2013). This quote highlights the brain's ability to continuously learn and build memories through perception and interaction with the surrounding environment.

The acquired information is not immediately consolidated in the memory; instead, at first, it is unstable and only following a stabilization process, called consolidation, the mnemonic trace becomes immune to interference (Dudai & Eisenberg, 2004). Sleep is essential in this process, as during it, information is spontaneously reactivated and transferred to neocortical areas, facilitating the integration of memory (Rasch & Born, 2013).

However, not all memories benefit equally from sleep; rather, emotionally relevant stimuli, rewarded information, and intent or instruction to remember are particularly favoured (Davidson et al., 2021).

Although these reactivations occur spontaneously during sleep, they can also be induced by the presence of external stimuli (such as smells or sounds), especially in the early stages of sleep, thus contributing to the strengthening of the memory trail (Knötzele et al., 2023).

Recently, some studies have shown that a multisensory approach to learning enhances content acquisition (Chierichetti & Tombolini, 2023) and that sleep following learning improves the retrieval of information, especially in school contexts (Cabral et al., 2018), thus constituting a means of

[1] The manuscript is the result of a collective work of the authors, the specific contribution of which is to be referred to as follows: introduction, paragraphs 2.1, 2.3, 3.1, 3.2 are attributed to Elisabetta Tombolini; paragraphs 2.2, 3.1, 3.3, 3.5 and conclusion are attributed to Elèna Cipollone; paragraph 3.4 is attributed to Stefania Morsanuto; Francesco Peluso Cassese is the Research Supervisor.

improvement for the learning process (Vidal et al., 2022).

Therefore, the targeted reactivation of memory during sleep, through the presentation of the same stimuli during learning and the subsequent sleep, is configured as a tool able to guide the consolidation of contents in the sleeping brain.

2. Theoretical background

2.1 Olfactory stimulation

Environmental enrichment stands as a pivotal scientific paradigm that enables us to deeply comprehend the intricate interactions between genetic factors and the surrounding environment. This concept is strictly related to brain plasticity, an extraordinary feature of the brain that allows it to adapt and alter its structure and functionality in response to environmental experiences and stimuli (Kempermann, 2019).

A crucial aspect of this gene-environment interaction involves the olfactory system, which constitutes a significant exception among sensory systems. The olfactory system possesses direct access to the limbic system, a brain region pivotal for memory and emotions. This significantly contrasts with other sensory systems, which necessitate the thalamus as an intermediary to reach this region.

In the specific context of olfactory enrichment, involving regular exposure to a variety of individually presented odours, a phenomenon of great interest emerges: neurogenesis. Scientific studies have unveiled how exposure to essential oils holds the potential to modulate human brain structure. For instance, recent research has shown that olfactory enrichment can lead to an increase in the volume of the olfactory bulb, a critical brain area for smell processing (Filiz et al., 2022). Additionally, an augmentation of grey matter in the hippocampus and thalamus has been observed in response to olfactory enrichment (Gellrich et al., 2017; Han et al., 2021).

Equally significant is the positive impact of olfactory enrichment on cognitive functions. Numerous studies have demonstrated that this form of stimulation can positively influence various cognitive areas. For instance, olfactory memory can be significantly enhanced through exposure to a diverse range of smell. Furthermore, olfactory enrichment has been associated with an improvement in verbal fluency and attention functions, particularly evident in patients with dementia (Cha et al., 2022).

In essence, regular olfactory exposure serves as a potent tool for enhancement cognitive functions and the quality of life. This phenomenon underscores the remarkable adaptability of the human brain and its capacity to actively respond to environmental stimuli. Olfactory enrichment thus proves to be not only a key to better understanding its functioning but also a potential intervention area to improve cognitive functions and quality of life. Such improvement also occurs when smell presentation takes place at night (Woo et al., 2023).

2.1 Declarative memory

Declarative memory is the type of memory that involves intentional and conscious storage of specific information, such as facts, events, concepts, and general knowledge (Coray, Quednow, 2022). Its development in children is a complex process that involves neurobiological and cognitive changes over time (Noh et al. 2023). At birth, not all neural structures are fully formed, which explains why not all memory systems are already present and developed.

The hippocampus, a key brain region, plays a fundamental role in the formation and recall of memories, especially in declarative memory, which includes both episodic and semantic memory (Rolls, 2023). The hippocampus is known for its contribution to the consolidation of long-term memories and spatial navigation. Its development occurs during childhood and adolescence.

From the very early moments of life, the hippocampus is present but continuously growing and maturing. During the first few years of life, the hippocampus continues to expand and establish new neural connections in response to sensory experiences and learning.

According to literature, the declarative memory system is partly present as early as 2 months of age, progressing to long-term information retention. Around 2 years of age, children can remember information for at least a day, and by the age of 3, they can retain such information for about a week. Unlike newborns, they demonstrate greater speed and flexibility in stimulus retrieval due to an increased ability to generalize information (Morgan Short et al., 2023; Godfrey et al. 2023). In general, the development of declarative memory in children is influenced by a combination of biological, cognitive, emotional, and social factors.

2.3 The role of sleep

In general, the development of declarative memory in children is influenced by a combination of biological, cognitive, emotional, and social factors.

Throughout the day, people are exposed to numerous stimuli, and only the consolidation process will determine whether those stimuli will remain in our memory or not. Thus, memory consolidation is the gradual stabilization of short memory content into long-term memory, as well as the phase during which this stabilization takes place (Dudai, 2004). Sleep plays a crucial role in memory consolidation: this process starts with the reactivation, during sleep, of recently encoded information present in neural memory (Stanyer et al., 2023; Vaseghi et al. 2022; Rasch & Born, 2013).

The fact that sleep optimizes and has a positive effect on declarative memory consolidation constitutes a valuable tool for improving learning and education. It has been shown that naps lasting more than 30 minutes after a lesson promote the retention of presented content (Capra et al., 2018).

However, only a small portion of the immense amount of information gathered through the senses is considered relevant by the brain and benefits from sleep, entering long-term memories. This includes emotionally salient memories (Tyng et al, 2017) and those relevant for the future or tied to an expectation of an upcoming test (Wilhelm et al., 2011).

Reactivation occurs spontaneously, but it can also be induced using external stimuli, such as olfactory ones. The presentation of a smell previously encountered during a learning session and then presented again during subsequent sleep, stimulates the content to be consolidated in the sleeping brain, leading to targeted reactivation (Neumann et al., 2020; Vidal et al., 2022; Knötzele et al., 2023). Particularly, studies have shown that only re-exposure to the smell during slow-wave sleep (SWS), characteristic of the first cycle or the first 40 minutes, contributes to the consolidation of hippocampus-dependent declarative memories, contrary to olfactory stimuli presented during the REM phase, which produced no effect (Rasch et al., 2007). Thus, the smell, used as a secondary sensory stimulus associated with learning content during the previous encoding period and as a cue for the reactivation of hippocampal activity during consolidation processes, leads to targeted memory reactivation (TMR) (Whitmore et al., 2022). Once reactivated, the content of hippocampal short-term memory is integrated into pre-existing networks located in the neocortex. The TMR effect offers an efficient way to optimize

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learning and memory processes. The amount of information that enters our senses extends by far our memory capacities. There's a need of select relevant information, worth being memorized, from irrelevant information that can be forgotten. The criteria for this selection are only partly understood. The TMR effect is a fascinating tool to gain some more influence on this selection. The literature about this topic on children is poor.

3. Research Project

3.1 Research Hypothesis and sample

The research hypothesis of this pilot study is to assess whether olfactory reactivation, presented during the afternoon nap phase to 2- and 3-year-old children, improves the consolidation of information, when the same smell was introduced during an educational activity.

The sample consists of 18 children aged between 2.1 and 3.8 years, equally divided between males and females, attending 4 facilities located in the Lazio region of Italy. In the subject recruitment process, inclusion criteria were as follows: (1) completion of the second year of age; (2) attendance at the nursery, including during the afternoon hours, as it was necessary for the nap routine; (3) developed language ability.

3.2 Methodology and instruments

The sample was randomly divided into two groups, an experimental group (n=13) and a control group (n=5), and the research project was based on a comparison between these two groups. The educational activity assigned involved a naming task, where children were required to learn the names of 24 animals associated with the presentation of visual stimuli.

The protocol was structured as reported below (Figure 1): before the training began, each individual child was asked to name both sets of visual stimuli, which consisted of animal's images, in order to assess the initial level of knowledge. Then, for two consecutive days, the same educational activity was presented to the classroom group, which involved naming the first set of 12 visual stimuli. In the experimental group, an additional olfactory stimulation was provided using the same lavender scent, through an ambient diffuser, both during the learning session and during the afternoon nap routine of 1 hours and half. At the end of the two days of training, a test was administrated, in which individual children from both groups were asked to name the visual stimuli from the first set, in order to assess their memorization. Children in the control group participated in the same educational activity but did not receive any olfactory stimulation during the learning session or the afternoon nap. The same training was repeated for an additional two days with the presentation of the second set of stimuli, and a different olfactory stimulation (orange scent) was provided to the experimental group. Again, a test was conducted at the end of this phase. One week after the first day of training, a long-term test was conducted, asking individual children from both groups to name all the visual stimuli to assess their acquisition of terminology over time.

The activities were presented by the reference educators of each group, to whom specific instructions were not given regarding the conduct, as the purpose was to investigate whether olfactory environmental enrichment, proposed both during the activity and during the afternoon nap, improved the acquisition and consolidation of information in memory, regardless of teaching style.

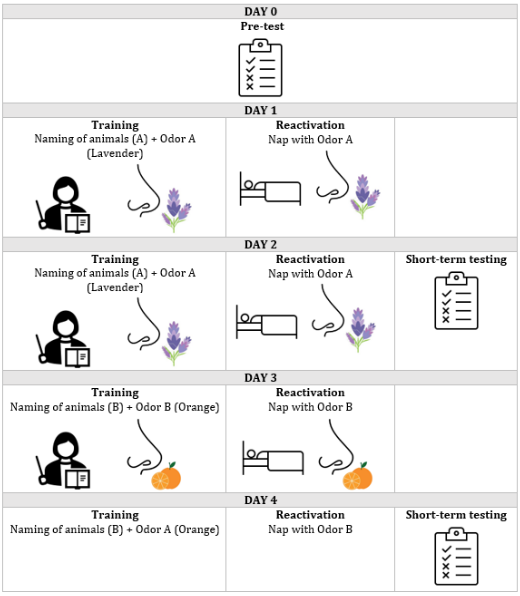
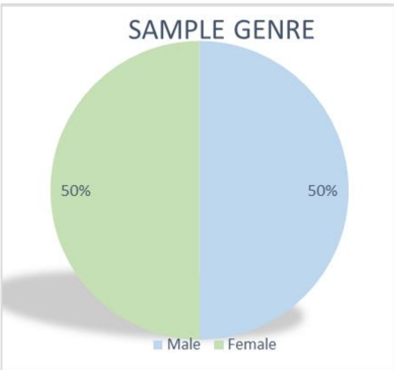
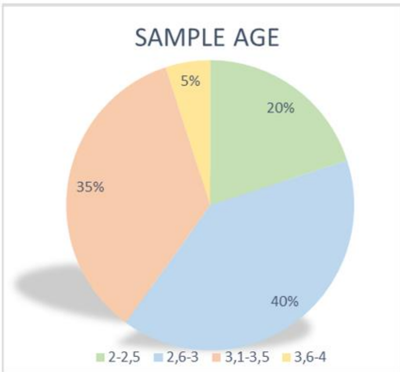


Figure 1. The research protocol

3.3 Results



Graph 1. Sample genre



Graph 2. Sample age

The sample consists The sample consists of 50% females and 50% males, attending 4 facilities located in the Lazio region of Italy (Graph 1), and the sample has an age range between 2 years and 4 years, with an average age of 2 years and 9 months (Graph 2).

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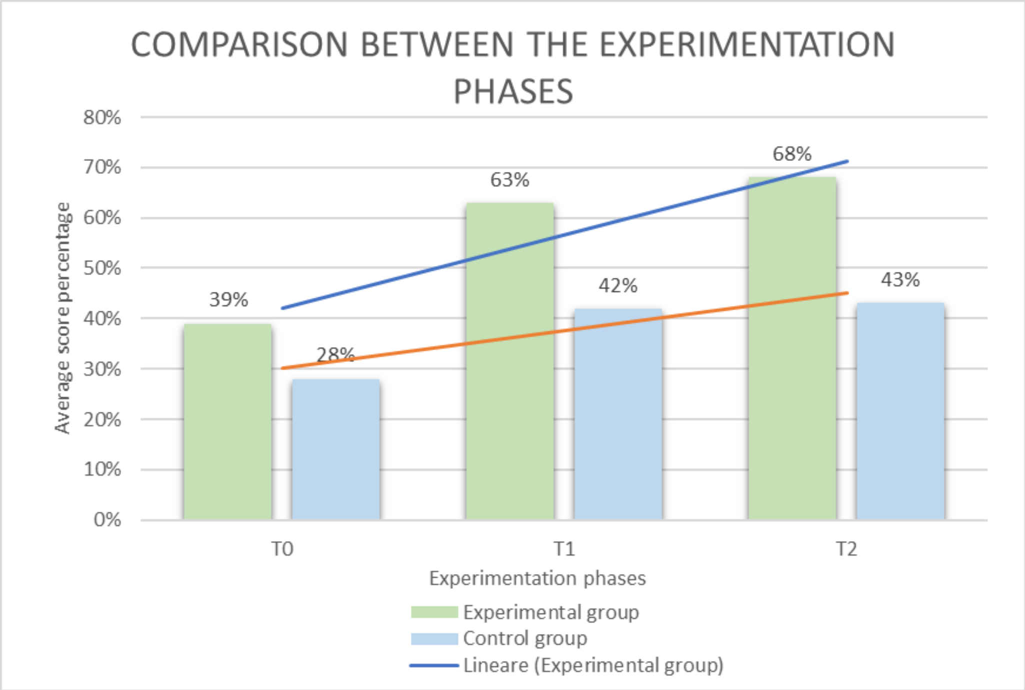
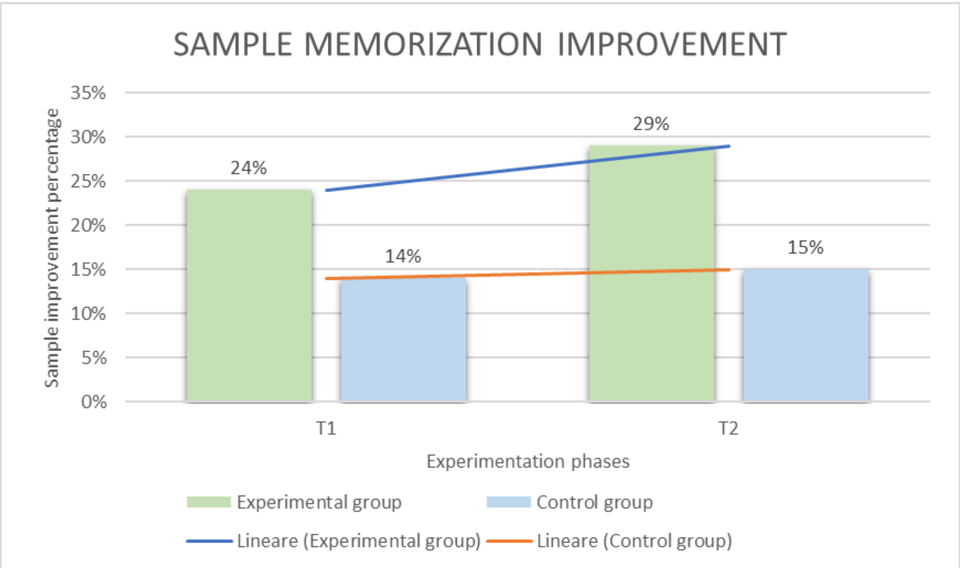


Graph 3. Comparison between the experimentation phases

In Graph 3, the comparison between the control group and the experimental group is presented in terms of the scores obtained throughout the entire project. The Graph illustrates how the baseline knowledge level of the presented stimuli was relatively uniform in both groups at T0 and was quite low in both experimental and control groups. At T1, noticeable differences in test scores begin to emerge, with the experimental group correctly responding in 63% compared to 42% in the control group. In T2, these differences amplify: the experimental group shows correct responses in 68%, while the control group only shows 43%.



Graph 4. Sample memorization improvement

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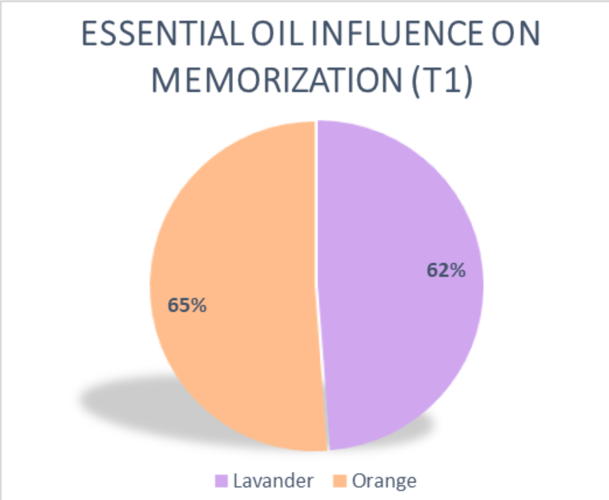
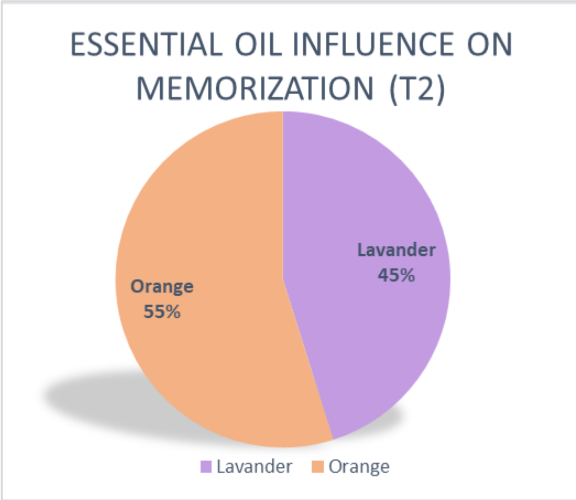
In Graph 4, there's showed the improvements observed in the two groups during phase 1 and phase 2. As can be observed, the experimental group exhibits a significant improvement already in phase 1 (short-term memory) of 24%, followed by further enhancement in phase 2 (long-term memory) of 28%. In contrast, the control group demonstrates a lower improvement in phase 1, at 14%, without showing significant improvement in phase 2, only at 15%.

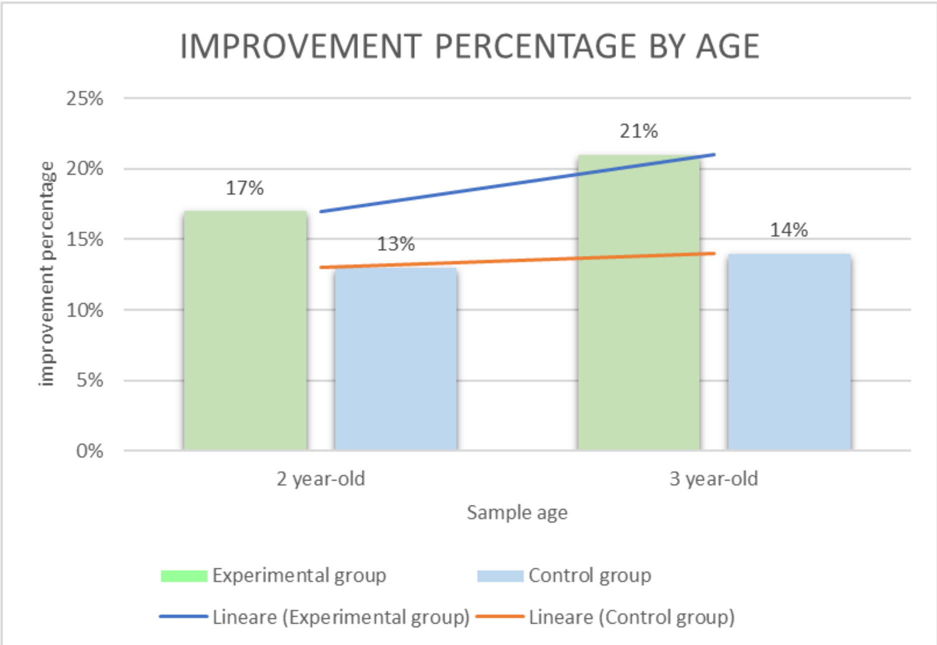


Graph 5. Essential oil influence (T1)



Graph 6. Essential oil influence (T2)

These two graphs (Graph 5 and Graph 6) illustrate the influence of the two selected essential oils on the memory process. As apparent, there are no substantial differences in the use of the two oils.



Graph 7. Improvement percentage by age

Finally, in Graph 7, the improvement percentages of the two groups are illustrated, divided according

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to the age groups, based on the differentiation of the memory development as provided by the literature. Consistent with the literature, children aged 2 show a smaller improvement in both groups.

3.4 Data analysis

Subsequently, data statistical analysis using SPSS was conducted. Since the sample is small (less than 25 statistical units per group), the Normality test is conducted, which is a prerequisite for performing statistical tests.

Tests of Normality

	Group	Kolmogorov-Smirnov Statistic	df	Sig.	Shapiro-Wilk Statistic	df	Sig.
T0	Control	.220	5	.200*	.913	5	.485
	Sample	.130	13	.200*	.969	13	.882
T1	Control	.230	5	.200*	.896	5	.390
	Sample	.144	13	.200*	.977	13	.959
T2	Control	.236	5	.200*	.881	5	.313
	Sample	.206	13	.137	.914	13	.211

*. This is a lower bound of the true significance.

Table 1. Tests of Normality

Table 1 presents the results of two normality tests, the Kolmogorov-Smirnov test and the Shapiro-Wilk test. The Shapiro-Wilk test is more appropriate for small sample sizes. For this reason, the Shapiro-Wilk test as a numerical means to assess normality was used.

From the table, it can be observed that at Time T0, T1, and T2 for both the control group and the sample, the dependent variable, Test Result, is normally distributed. In fact, the p-value (Shapiro-Wilk test value) is greater than 0.05, indicating that the data can be considered not deviating from a normal distribution. The Shapiro-Wilk normality test has revealed that the dependent variable for both the control and experimental groups is normally distributed.

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means					95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
T0	Equal variances assumed	.726	.407	1.088	16	.293	2.585	2.375	-2.450	7.619
	Equal variances not assumed			.896	5.375	.408	2.585	2.883	-4.674	9.844

Table 2. Independent Samples test

Displayed here (Table 2) are the results of the independent samples t-test. In our case, the null hypothesis suggests that the average of the experimental group and the control group are the same, while the alternative hypothesis suggests that they are different.
The Levene's test has a p-value > 0.05 (Sig.), indicating that the homogeneity of variances test is passed. Therefore, the first row of the table is evaluated.
The table also shows that the bilateral p significance level is > 0.05 - Sig. (2-tailed). This implies rejecting the alternative hypothesis and not rejecting the null hypothesis. The fact that the null value is within the confidence interval (Lower - Upper) strengthens this hypothesis.

Thus, the control group and the experimental group can be considered homogeneous.

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
T1 - Total	Equal variances assumed	3.076	.099	2.247	16	.039	5.077	2.259	.287	9.866
	Equal variances not assumed			1.714	4.918	.148	5.077	2.963	-2.577	12.731
T2 - Total	Equal variances assumed	3.725	.072	2.659	16	.017	6.031	2.268	1.224	10.838
	Equal variances not assumed			2.020	4.897	.101	6.031	2.986	-1.693	13.754

Table 3. Independent Samples test

At this point, there's a need of investigate if there are statistically significant differences between the two groups at instants T1 and T2 (after the use of essences).
Once again, the Levene's test has a p-value > 0.05 (Sig.) for both instants (T1 and T2), indicating that the homogeneity of variances test is passed. Therefore, the first rows of the table are evaluated.
The table also shows that the bilateral p significance level is < 0.05 - Sig. (2-tailed). This implies rejecting the null hypothesis and not rejecting the alternative hypothesis. The fact that the null value is outside the confidence interval (Lower - Upper) reinforces this hypothesis.
Specifically, at time T1, the experimental group obtained a mean superior to the control group by 5.077 points (short-term memory), and this result is statistically significant; at time T2, the experimental group obtained a mean superior to the control group by 6.031 points (long-term memory), and this result is statistically significant.

3.5 Discussion

In this pilot study, the impact of using olfactory stimulation was examined during both learning and sleep phases on memory retention in a representative sample of children aged 2 and 3 years.
The results have highlighted very promising data. In T1, evaluated after only 2 days of training, the experimental group has corrected responses in 63% of cases, compared to 42% in the control group. This difference amplified in T2, conducted after one week, where the experimental group achieved correct responses in 68% of cases, while the control group achieved correct responses in only 43%. This suggests that the smell triggered hippocampal reactivation of the learning content.
Therefore, olfactory stimulation, regardless of the specific scent (as there were no significant differences observed between the two selected scents), led to a significant improvement in memory retention of the presented stimuli in both learning phases. Consistent with the literature, age-related differences were observed in information acquisition. Despite younger children retaining less information, they still greatly benefited from the methodology compared to the younger children in the control group.
This improvement was consistent across the three selected facilities. It was not requested educators to adopt specific educational styles, as the aim was to observe the validity of this methodology regardless of them. Results demonstrate that despite differences in educational styles, the methodology's effectiveness was universal.
Statistical analysis revealed that the results obtained were statistically significant in terms of variance

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homogeneity and the difference between the groups means in T1 and T2.
It is evident that this is only a pilot study, but it is crucial to replicate the research on a larger sample to determine the existence of a relationship between the variables under examination and also extend the duration of the experimental protocol to observe the impact over a longer period.
In line with the literature (Gellrich et al., 2017; Han et al., 2021; Woo et al., 2023), a strong relationship has emerged between external stimulation during sleep, in this case olfactory stimulation, and the enhancement of memory retention, thanks to the close neural connection between the hippocampus and the olfactory system. Building upon these premises, the present research has demonstrated consistency with the literature (Neumann et al., 2020; Vidal et al., 2022; Knötzele et al., 2023) regarding the influence of olfactory stimulation, both during the learning phase and subsequent sleep, in mnemonic enhancement, leading to TMR.
Previous studies (Whitmore et al., 2022; Rasch et al., 2007) had focused on an adult sample, while in this context, the focus was shifted to a pediatric sample, where sleep plays a central role in promoting the enhancement of experiences and learning. The TMR effect has proven to be a powerful tool for optimizing learning and memory in infants, where the quantity of information they encounter is substantial.
Consistent with the literature, the TMR effect can thus become an aid in selecting relevant information that needs to be memorized, distinguishing it from irrelevant information that can be forgotten. This applies not only to adults but also to children.

Conclusion

Every day, people are exposed to numerous stimuli, and the brain must work hard to select which ones are relevant to us. In young children, during their growth phase, this selection is even more critical, as their minds are in a crucial stage of development and learning. The approach of using specific odors to enhance memorization, acting on the hippocampal level, offers a fascinating opportunity to support the cognitive development of children. The hippocampus, a key brain region involved in memory formation, seems to be particularly sensitive to olfactory stimuli. The ability of odors to evoke memories and influence emotional states has long been known, but the idea of using them strategically to improve memorization is an innovative and promising concept.
This research serves as a pioneering exploration into the realm of enhancing memorization in young children through olfactory stimuli intervention. The noteworthy findings reveal that improvements in memory were consistently observed, irrespective of the specific fragrance employed. This suggests a wide-ranging and adaptable applicative potential for the methodology.
The versatility of this approach is particularly striking, demonstrating its efficacy across diverse contexts, age groups, and educational settings. The methodology's successful adaptation to varying teaching styles within nursery environments underscores its flexibility and applicability.
In essence, this research marks a significant milestone in redefining early learning methodologies for children. The deliberate use of olfactory stimuli as a catalyst for enhanced memorization presents an innovative opportunity that holds the promise of a lasting impact on cognitive development and learning in children. While it's acknowledged the preliminary nature of this investigation, the prospect of future studies in a more expansive context offers an exciting glimpse into the potential evolution of this methodology in shaping the landscape of education and child development.

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