ORIGINAL ARTICLE

Possible Role of the Pineal Gland in the Human Memory System

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Abstract

Purpose: Pineal Gland (PG) is a midline brain structure and part of the epithalamus, a dorsal posterior segment of the diencephalon. Most findings on the role of this structure in brain function are relevant to melatonin secretion, and only a few reports are available on its involvement in brain cognition.

Materials and Methods: Due to some suggestions on the role of the diencephalon areas, including the PG, in the human memory system, we used data from two previous MRI studies on 32 and 295 healthy individuals, in order to identify first, if the PG shows activations in fMRI and during a memory retrieval task as well as showing functional connectivity with other brain structures, and second, if there are any associations between the PG volume and the memory scores of the individuals.

Results: Using a standard PG atlas, our results showed significant activations in PG during memory retrieval, with the strength of these activations increasing with the increment of the cognitive load of the task. Also, PG showed functional connectivity with other brain structures during fMRI, the pattern of which also changed with the cognitive load of the retrieval. Finally, the volume of the PG showed significant associations with the scores of the memory tests.

Conclusion: Our knowledge of the PG still needs improvement, and we hope our findings here could be a help for that as well as a help to better understand the mechanisms of memory storage and retrieval in humans.

Keywords: Pineal Gland; Cognition; Magnetic Resonance Imaging; Memory; Brain Volume; functional Magnetic Resonance Imaging.

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1. Introduction

The Pineal Gland (PG) is an interhemispheric neuroendocrine organ that, as a small canonical gland of about 100 mm^3, is medially located in the epithalamus of the vertebrate brain along with the habenula nuclei, and is surrounded by some structures such as the thalami, posterior third ventricle, and the splenium of the corpus callosum [1]. The PG, habenula, and stria medullaris together form the epithalamus, a prominent structure that overlies the thalamus [2]. The PG reaches full development approximately at the age of 2 years, and although there are reports on the consistency in its weight and size in late life [3], there are reports that its volume is correlated positively with the melatonin level [3]. In a previous study, it was reported that the volume of the PG declines with age [4].

A set of features has made this endocrine tissue influential and crucial. This circumventricular organ, as a whole apparatus, concludes mechanisms for secretion (into the bloodstream and CSF) and synthesis of the indoleamine melatonin (N-acetyl-methoxytryptamine)-as the most identified one-, as well as the arginine, vasotocin, serotonin, and some forms of the neurosteroids [5]. The PG also regulates the circadian rhythm which is mainly mediated by the released melatonin [6]. Some other physiological functions in which the PG is reported to be involved include aging [7], modulating gonadal activity [8], mood [9], sexual maturation and reproduction [10], seasonal reproduction [11], mediating responses to light and altering pigment coloration [8], and sleep regulation [12].

There are also reports on the association of the PG's malfunction with some disorders, including obesity [13], hypertension [14], cancer [15], bipolar disorder [16], diabetes [17], and sudden infant death syndrome [18]. In addition, the PG volume is reported to be lower in patients with insomnia [19], schizophrenia [20], Alzheimer's disease [21], Attention Deficit Hyperactivity Disorder (ADHD) [22], obsessive-compulsive personality disorder [23], obesity [22], and sleep problems [24].

The PG has generated much speculation about its functional role. Its unique characteristics, its prominent location, and its anatomical appearance have awakened the interest of many researchers, scientists, and even philosophers. Since ancient times this enigmatic organ, as it was called by Van Gehuchten, has been attributed an outstanding role as the connection between the spiritual and material worlds in human beings. Also, according to ancient Indian traditions, humans would be equipped with a ''third eye'' or mystical organ (the PG), corresponding to the sixth chakra, which would provide a window into the spiritual life of individuals and would enclose the key to its mental power [25]. One of the most outstanding proponents of the role of the PG was René Descartes, who put this organ as the physical seat of the human soul; the PG was also responsible for the intimate mechanism controlling the precise operation of the human body, and thus being involved in mental disorders [25]. All these, in addition to its complicated location in the brain, its connections with the adrenal cortex [13], its functional connections with the hypothalamus and pituitary glands, and its forms of the prevalent cancerous states [6] are the factors that signify studying the functions of the PG in the brain; however, our current knowledge of the role of the PG is mostly focused on its physiological roles and melatonin secretion, and there is little information available on its cognitive roles.

There are rare reports available on the role of the PG in brain cognition. In one study, together with other brain regions, PG exhibited significant activations during the meditation process, supporting the long-lasting thought that PG plays an important role in intrinsic awareness [26]. Due to the very limited information available on the PG's role in brain cognition, we decided to perform a series of studies to identify its other possible roles. Five of those previous works on this topic are finalized, and the current study is the sixth.

In the first study and using a rational approach, we hypothesized that the PG may be showing roles in the human memory function [27]. In the second study, to have access to the exact location of the PG in the MRI scans of the human brain, we decided to develop an atlas of the PG in the standard space [1], based on the MRI data of 152 healthy young individuals. Next, using that PG atlas, and collecting data from 301 individuals in the age range of 19 to 76 years old [28], we performed the volumetry of the PG and estimated the average volume of the PG for the individuals in the different age ranges [4]. We continued by assessing the association of PG's volume with some cognitive abilities of the brain and observed that it was significantly associated with the scores of individuals on the Block Design test, Symbol

Digit Modality Task, and Discriminative and Choice Reaction Time. In the fourth study, we designed a novel fMRI task by which we could identify the brain networks involved in the retrieval of long-term memory (LTM) [29]. In that work, the hypothesis was that the brain areas that show an increased activation when the load of the LTM retrieval increases should be responsible for the function of interest. In the fifth study, we reviewed our current knowledge of the human LTM mechanism based on the Principles of Neural Science book [30], and we declared that our current knowledge has many shortages in explaining this mechanism. Here, and in the sixth study, we aim to use the data we collected in our previous studies to investigate any possible role of the PG in the memory function. We are aware that an association does not necessarily represent causality; however, observing an association could be an initial evidence for such a role, as well as a strong suggestion for performing further works in the future to more precisely test the validity of the hypothesis.

There are some previous findings that could be the basis of our hypothesis on the involvement of the PG in the memory function. For example, the brain structures near the PG have shown involvements in memory, such as the mammillary bodies, which were observed active in memory tasks. These data are consistent with the notion that mammillary bodies contribute to memory via their projections to the anterior thalamic nuclei [31]. Habenula, one other nearby structure, is thought to have evolved in close association with the PG and they have reciprocal connections [2]. Habenular nuclei lesions have been shown to impair memory function [32], and the involvement of the LHb in spatial memory had previously been suggested by the findings that lesions of the habenular complex in rats altered learning and retrieval in the WM [33]. Also, there is evidence that each of the constituent neural structures of the Papez circuit contributes to memory, and lesions of the circuit of Papez disturb the organization and recall of memory [31]. Finally, damage to the structures that bear an intimate relationship to the PG, including the hippocampus, mammillary bodies, anterior thalamic nuclei, and cingulate gyrus (retrosplenial cortex) [35] can result in anterograde amnesia. One study suggested that the medial temporal lobe and medial diencephalon, comprising the hippocampus and mammillary bodies, have been implicated in event memory, but there remains much uncertainty about how these brain regions interact to support this function [31], and this study suggested that uncovering the role of new brain areas need to consider a wider network of structures that form the neural bases of episodic memory.

As a result, in this work, using an MRI atlas of the PG, using data from a previous task-based fMRI study on LTM retrieval, using structural MRI data, and using data on the memory tests of the individuals, we aim to study any possible role of the PG in the human memory function. Any finding here would be considered as some initial findings that need replication and further confirmations.

2. Materials and Methods

2.1. Participants

This study used data from two previous works [4, 28, 29], and both works were part of the Iranian Brain Imaging Database (IBID). In the first study, we used data from 32 healthy young individuals (18F), with a mean age of 30.16 ± 6.4 (20-39 years old), and a minimum of 14 years of education [29]. Our second study included 295 MRI data from healthy individuals in the age range of 19 to 76 years old [4]. We used the data of the first study to evaluate the PG's activation as well as its functional connectivity during a memory task in fMRI, and the data of the second study for estimating the associations of brain structures with the memory scores of the individuals. Both these works were part of the Iranian Brain Imaging Database (IBID), which had the aim of developing a normative neuropsychiatric database of the human brain [28].

The inclusion criteria in both works were based on the IBID, with the aim of excluding any individual with drug or alcohol consumption, internal or neurologic diseases, long-term or current use of medications, any history of chronic headache, tinnitus, dizziness, seizure, or nausea, family history of any neurologic disease, any surgery with anesthesia, history of losing consciousness or head trauma, or any metal objects in the body [36]. Each participant was examined by a physician for blood pressure, heart and respiratory rates, and a neurological examination including vision and hearing. To assess mental health, the Depression Anxiety Stress Scales (DASS-21) [37] was administered, which was normalized for the Persian language and population [38]. As stated in the original study, all participants successfully passed the mental and physical health assessments. The

participants provided written informed consent, and the ethics approval was provided for the study (approval No. IR.NIMAD.REC.1396.319) by the Iranian National Institute for Medical Research Development.

2.2. Memory Tests

The memory function of all participants was assessed in the IBID study and in two general domains of Episodic Memory and Working Memory. I) Episodic Memory: Rey Auditory Verbal Learning Test (RAVLT) [39, 40] was used. This test measures the abilities in attention and learning, and specifically several aspects of memory [41]. The RAVLT test is normed for the Persian population and language [42]. The second test in this category was the Benson Complex Figure (Copy, Delayed Recall, and Recognition) [43, 44]. This test is a simplified version of the Rey-Osterrieth figure test, assessing visuo-constructional and visual memory functions, and includes a copying, a delayed reconstruction (after 10-15 minutes), and a recognition phase [45].

II) Working Memory: Forward and Backward Digit Span tasks, as a subtest of the Wechsler Memory Scale Revised test, were used [46, 47]. In this test, the subject is asked to recall the items just after the examiner reads them aloud, in the order (Forward) or in the reverse order (Backward). The Forward Digit Span test relies on the phonological loop capacity, and the backward test needs the ability to manipulate while maintaining the information, therefore it relies on the central executive function [48]. The second test in this category was the one-Back version of the N-Back task [49]. In this test, a number of digits are randomly presented to the participant only once, and the participant is asked to indicate if the currently presented digit matches the previously presented one. We used a Persian-normed version of this test [50].

2.3. The fMRI Task

As detailed in the first study [29], two categories of images (high load and low load) which were different in the number of items to memorize, the complexity of the episode to memorize, and the number of colors in the images, were selected. Twelve categories of images relevant to daily events were selected, and each category included one high- and one low-load image. The images are illustrated previously [29]. The images were electronically sent to each individual 14 days before the fMRI test, and the participant was asked to look at the images as many times as possible until he/she could memorize all the details of each image. Two days before the test, the participants were asked not to look at the images any further, and therefore they had 12 days to memorize the images and had two nights of sleep (consolidation phase) before their fMRI test.

The participants were asked to retrieve an image once its name (cue) was read to him/her during the fMRI scan and to review all the details of the images in the shortest time. There were 24 trials in the task, relevant to the 24 images, and the task lasted for 17:45 minutes (355 fMRI volumes). In two steps of the trial, the participant used the MR-compatible response keys: i) when retrieving an image finished the participant pressed a particular key to inform us of the time of that; ii) to give a score to the quality of the retrieval, the participant used one of the four keys to rate his/her retrieval as weak, moderate, good, or excellent. In summary, the aim of designing this task was to activate the memory-related brain areas and also to modulate the activation of those regions by changing the level of the cognitive demand of the task. Further details of the fMRI task have been published previously [29].

2.4. Imaging

The MRI machine in the IBID study was a Siemens 3.0 Tesla scanner (Prisma; Siemens Healthcare GmbH, Federal Republic of Germany; Production: 2016), and using a 64-channel head coil the functional T2*-weighted images were collected using blood oxygen level-dependent (BOLD) contrast. A three-dimensional T1-weighted anatomical scan was also acquired before the EPI scan. MR-compatible headphone and response keys were also used during the scan, and the headphone volume was set to a comfortable level before the scan.

2.5. Pineal Gland Standard ROI

None of the currently available brain atlases has provided the standard masks for the PG. This shortage was the incentive of a previous work, in which we built a standard atlas for the PG in the MNI space [1]. As a brief introduction to that work, we used 152 (53F) three-dimensional T1-weighted MRI data (3T, Siemens, PRISMA) of healthy young (20 to 30 years old) individuals, all right-handed, mentally and physically healthy, and with at least 14 years of education. The strict

inclusion criteria were based on the IBID study [28]. First, the PG was manually delineated on all MRI data, using two experts. After checking the reliability of the delineated contours by estimating the Intraclass Correlation Coefficient (ICC) between the two contours, the ROIs from rater1 were used for atlas preparation, and the ROIs from rater2 were used for assessing the accuracy of the outcome atlas. Using the FSL Brain Extraction Tool (BET), the non-brain tissues were removed from the T1-weighted images, and a local brain MRI template was constructed using an iterative routine similar to those explained in previous works [51]. Finally, two versions of the PG atlas were provided; one by transferring the ROI masks (from rater1) to the local template, and the other by performing the same procedure to the MNI standard space. Another version of both atlases was also provided, by thresholding the probability maps of the PG atlases, to remove the voxels with a probability lower than 0.15. In the current study, we used both original and thresholded versions of the PG atlas in the MNI space, as illustrated in Figure 1.

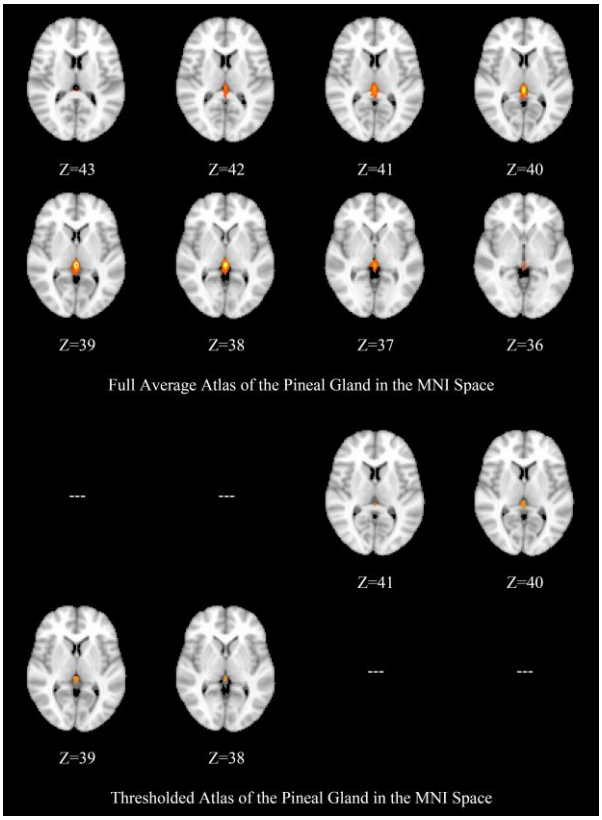

Figure 1. The pineal gland standard atlas that we used in this work; the full-average atlas is the original not-thresholded atlas, and the thresholded atlas only includes the voxels which have a probability of over 15% of being the pineal gland. The atlases are illustrated in the axial view, and only the brain slices which do include the pineal gland are shown

2.6. fMRI Data Analysis

2.6.1. Preprocessing

Initially, a visual quality check was performed in order to spot possible macroscopic artifacts, vibration/motion evidence, head tilt and head positioning, signal loss, ghosting, or other possible artifacts in the data. No data were excluded in this step.

The analysis was performed using FEAT (fMRI Expert Analysis Tool), part of FSL (FMRIB's Software Library, v. 5.0.9). Preprocessing steps included 1) motion correction using MCFLIRT, FSL (Motion Correction from FMRIB's Linear Image Registration Tool); 2) skull-stripping for removal of non-brain tissue from the structural T1-weighted images using Brain Extraction Tool (BET), FSL; 3) slice-timing correction (data acquisition: interleaved); 4) spatial smoothing, using a Gaussian kernel of FWHM = 5.0mm; 5) Melodic ICA data exploration, to identify remaining data artifacts, and to help to better explore activation in the data; 6) multiplicative mean intensity normalization of the volume at each time point; and 7) high-pass temporal filtering (Gaussian-weighted least-squares straight-line fitting, with sigma = 60.0s). 8) Normalization of the functional images to the standard Montreal Neurological Institute (MNI) brain atlas was also performed via i) co-registration of the functional images to the high-resolution T1-weighted scan, using FLIRT (FMRIB's Linear Image Registration) and the BBR (Boundary-Based Registration) cost function; ii) linear registration of the structural T1 images to the MNI space, with 12 DOF.

2.6.2. First-Level and Higher-Level Analyses

The statistical analysis was based on a general linear model (GLM) and was performed using FEAT (version 6.0.0), FSL. The FILM (FMRIB Improved Linear Model) pre-whitening was used for statistical analysis of the fMRI time-series, in order to make the statistical approaches valid and maximally efficient, which devoted a "z-score" to the corresponding BOLD signal. As explained above, registration of the estimated function map to the corresponding structural image and ultimately to the MNI space was carried out.

The group-level analysis was performed using FLAME (FMRIB's Local Analysis of Mixed Effects) in FSL, to estimate averages for each of the image groups, as well as to compare them. Cluster thresholding was performed

only to reveal the significantly active clusters. The criteria for identification of active clusters (in the average contrast) was a voxel-level probability threshold of z-value > 3.0; for estimating the contrast of the high and low conditions, the threshold was set as z-value > 4.0; False Discovery Rate (PFDR < 0.05) was used to correct for multiple comparisons.

Two regressors were defined in the analysis, corresponding to the high and low load conditions. Regarding the responses of the participants during the retrieval of the images, those stimuli which received a "weak" score for the success of the retrieval were excluded from the final EVs (explanatory variables) of the GLM analysis. Also, only the portion of the 15-second interval in which the participant was actively recalling the image (before pressing the key) was considered in the EVs (Explanatory Variables). The individual GLM analyses were performed by creating a boxcar function of tasks (different conditions) against rest, being convolved with a canonical hemodynamic response function and its temporal derivatives. Three contrasts were defined here: a) high-load images (average), b) low-load images (average), and c) high minus low contrasts. This analysis was initially performed at the whole-brain level, but in this work, our focus was only on the activations of the pineal gland in these 3 conditions; as a result, an ROI analysis was performed, using the standard mask of the PG, as explained above.

2.6.3. PG Functional Connectivity

Following the analysis of activation in the PG, we performed a functional connectivity analysis between the PG and 27 brain structures, separately for the right and left brain hemispheres. The 27 structures were based on the findings of the previous work [29] that showed the involvement of these areas in LTM retrieval. We performed this ROI-to-ROI functional Connectivity analysis using CONN Toolbox (v.18b; https://www.nitrc.org/projects/conn).

For the sake of preprocessing the original fMRI data, functional images were first subject to motion estimation and correction, then translation to center (0, 0, 0 coordinates), slice timing correction, ART-based (Artifact Detection Tools) identification of outlier scans (https://www.nitrc.org/projects/artifact_detect/), tissue segmentation, normalization to MNI space, and spatial smoothing with a Gaussian kernel (8mm FWHM). The structural images were also centered, segmented, and normalized to the MNI space.

A weighted GLM (general linear model) was then used to conduct first-level analysis. The functional connectivity of the PG with the ROIs was estimated using three groups of regressors: I) the mean of the high load condition; II) the mean of the low load condition; and III) the high versus low load condition. The connectivity estimation was based on the bi-variate correlation method using HRF-weighting. Prior to this step and in order to reduce the effect of noise, the resulting preprocessed images were band-passed filtered to 0-0.1 Hz; the effect of denoising was visualized here by illustrating the mean of the distribution of connectivity values for each subject. For the second-level analysis of connectivity, and for between-conditions distinction, the F-statistic test was used, with the significance level based on P-value < 0.001 (FDR corrected). In order to validate the multiple comparisons, significance tests were based on standardized Z-scores.

2.7. Associations of the PG Volume with the Memory Tests

In order to identify the possible role of the PG in the memory function, in addition to analyzing the PG's activation during a memory task in fMRI, we selected another approach by assessing the associations of the volume of the PG with the memory scores of the individuals. The memory scores are explained above, and the estimation of the PG volume was also explained in a previous work [4]. In that previous work, the PG volume was estimated in order to identify its associations with some factors such as age and gender, as well as with some cognitive measures other than memory; we are here using the PG volumes estimated in that work. The association of the PG volume with the memory scores was based on the Pearson's R correlation coefficients of the two values, estimated in MATLAB; multiple comparisons correction was performed here, using the Bonferroni correction method (p-FWER < 0.05).

3. Results

3.1. Cognitive Assessments

As stated in Methods, several memory tests were performed on the participants, and the average scores

of these tests are provided in Table 1. The results are relevant to the 11 measures of the RAVLT test (A1 = WS = first trial, A2 = DR= seventh trial, A3 = PIS = Proactive Interference Score, A4 = RIS = Retroactive Interference Score, A5 = FR = Forgetting Rate, A6 = PES = Position Effect Score, B1 = FAL = final acquisition learning, B2 = TL = total learning, B3 = LOT = learning over trials, C1 = NPS = net positive score, C2 = ROR = recognition over recall), 2 measures of the Forward- and 2 measures of the Backward-Digit span tests, 4 measures of the Benson complex figure test, and 8 measures of the N-back test. The scores of the individuals in the memory tests were later used for assessing the associations of the volume of the brain structures, including the PG volume, with the memory tests.

3.2. Pineal Gland Activation

Table 2 provides the details of the PG activation, in the three contrasts of the High (H), Low (L), and High > Low (H > L). The results are provided using both full-average and thresholded atlases. The maps of the activations are also provided in Figure 2.

In the full average atlas, the maximum percent signal change (SC) of the PG in the High and Low contrasts were 0.57% and 0.43%, respectively (effect size = 0.467). The maximum z-value was 4.99 in H, 4.55 in L, and 2.95 in H > L contrasts. The number of voxels with a significant z-value was also higher in the H condition, with 121 > 44

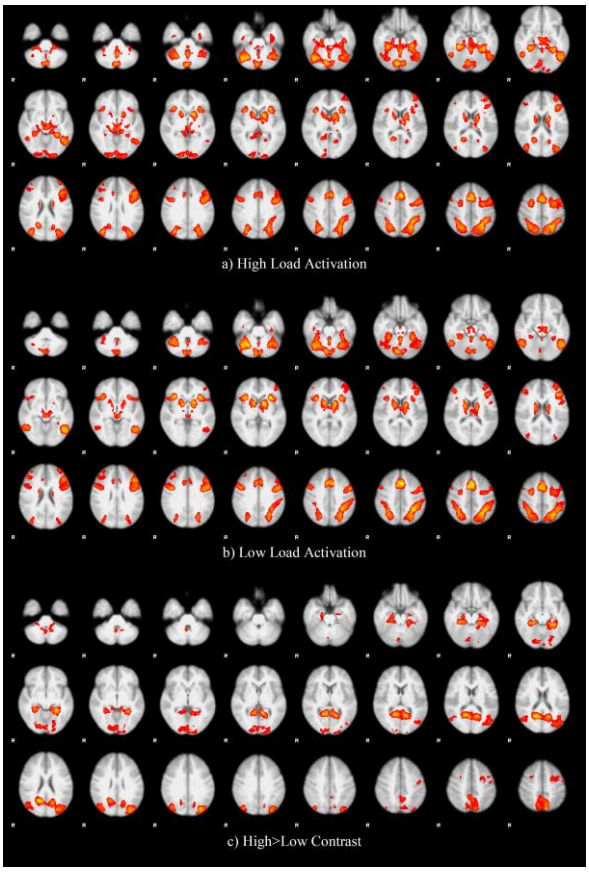

Figure 2. Brain activation maps in the three conditions relevant to a) High load, b) Low load, and c) High > Low load contrasts; these activation maps were later used at the ROI analysis step in order to extract the strength and extent of the activation of the PG in the three conditions. The ROI analysis was performed using both original and thresholded atlases of the PG

Table 1. The average scores (±standard deviations) of the individuals in the memory tests of interest; the tests included the Rey Auditory Verbal Learning Test (RAVLT), Benson Complex Figure (Benson), Forward and Backward Digit Span tasks (FDST & BDST), and the one-Back version of the N-Back task. These memory scores were used to assess the association of brain structures with the memory ability of the participants. M= male; F= female

Cognitive measures		M	F	Cognitive measures		M	F
RAVLT	A1	7 ±1.44	7.55 ±1.29	Benson	Copy Score	6.07 ±0.94	5.94 ±1
	A2	10.7 ±2.41	12.26 ±1.84		Copy Time	9.27 ±1.8	9.13 ±1.78
	A3	1 ±2.35	0.68 ±1.45		Recall Score	5.23 ±1.38	5.35 ±0.98
	A4	1.67 ±1.88	0.9 ±1.68		Recall Time	7.37 ±2.44	7.48 ±1.73
	A5	1.43 ±1.83	0.97 ±1.4	N-Back	True	110.57 ±20.58	112.39 ±10.36
	A6	0.27 ±3.96	0.65 ±2.85		Error	5.33 ±3.18	5.58 ±3.58
	B1	12.67 ±1.47	13.68 ±1.11		No	4.1 ±20.77	2.03 ±10.58
	B2	52.03 ±5.72	56.52 ±5.24		MinTimeRec	296.17 ±77.96	247.06 ±59.19
	B3	17.07 ±6.12	18.77 ±4.94		MaxTimeRec	1193.13 ±363.13	1180.19 ±330.27
	C1	12.4 ±3.18	13.87 ±1.45		AvgTimeRec	580.83 ±129.26	522.84 ±125.27
	C2	2.77 ±2.1	2.06 ±1.67		VarTimeRec	163.6 ±63.02	158.19 ±59.8
FDST	Longest	6.07 ±0.94	5.94 ±1		Percentage	92.07 ±17.22	93.52 ±8.59
	Total	9.27 ±1.8	9.13 ±1.78				
BDST	Longest	5.23 ±1.38	5.35 ±0.98				
	Total	7.37 ±2.44	7.48 ±1.73				

in z > 2.3, and 84 > 19 in z > 3.0. Also, 12 and 2 voxels with a z-value above 2.3 and 3.0 were also observed in the H > L condition, respectively. In addition, the mean z-value of the thresholded map with z > 2.3 was 1.35 in H, and 0.45 in L (effect size = 0.92), and for the map with z > 3.0, it was 1.03 in H, and 0.24 in L (effect size = 0.85).

In the thresholded atlas, the maximum percent signal change (SC) of the PG in the High and Low contrasts were 0.38% and 0.29%, respectively (effect size = 0.312). The maximum z-value was 3.43 in H, and 2.91 in L. The number of voxels with a significant z-value was also higher in the H condition compared to L, with 12 > 7 in z > 2.3, and 5 > 1 in z > 3.0. In addition, the mean z-value of the thresholded map with z > 2.3 was 0.73 in H, and 0.41 in L (effect size = 0.403), and for the map with z > 3.0, it was 0.35 in H, and 0.064 in L (effect size = 0.52).

3.3.Pineal Gland Functional Connectivity

We estimated the functional connectivity of the PG with 27 brain structures (separately for the left and right hemispheres), in the three conditions of High, Low, and High > Low. Estimations of the connectivity are provided in Table 3, and the illustrations are provided in Figure 3. The estimations here were solely based on the thresholded PG atlas.

In the low load condition, two brain structures showed a significant positive FC with the PG, including the right and left thalamus. On the other hand, there were five brain structures with a negative FC with the PG in the Low condition, including right and left postcentral gyrus, left superior parietal, left precentral gyrus, and right inferior lateral occipital. In the high load condition,

Table 2. The results of estimating the activation of the pineal gland in the fMRI task of the study, using both full-average and thresholded atlases of the pineal gland. Using each atlas, the PG activation was estimated at the high load, low load, and high > low conditions of memory retrieval. %S.C. = percent signal change; zstats = the z values of the fMRI activation; thresh_zstats = only the z values above the threshold (in parenthesis) are considered; #voxel = number of active brain voxels; max = maximum; std = standard deviation; X,Y,Z = coordinates of the voxel with the maximum activation in the standard space

Atlas	Contrast	Stats	#voxels	mean	max	std	X,Y,Z
Full Average Atlas	High	%S.C.	--	0.14	0.57	0.17	0,-40,6
		zstats	256	1.41	4.99	1.77	-2,-26,0
		thresh_zstats (2.3)	121	1.35	4.99	1.62	-2,-26,0
		thresh_zstats (3.0)	84	1.03	4.99	1.62	-2,-26,0
	Low	%S.C.	--	0.09	0.43	0.13	0,-38,4
		zstats	256	0.80	4.55	1.46	-2,-28,-2
		thresh_zstats (2.3)	44	0.45	4.55	1.08	-2,-28,-2
		thresh_zstats (3.0)	19	0.24	4.55	0.91	-2,-28,-2
	High>Low	%S.C.	--	0.05	0.30	0.06	4,-42,6
		zstats	256	0.69	2.95	0.88	4,-42,6
		thresh_zstats (2.3)	12	0.11	2.95	0.54	4,-42,6
		thresh_zstats (3.0)	2	0.02	3.17	0.26	-2,-40,4
Thresh. Atlas	High	%S.C.	--	0.16	0.48	0.15	0,-38,6
		zstats	40	1.53	3.41	1.25	0,-38,6
		thresh_zstats (2.3)	16	1.13	3.41	1.41	0,-38,6
		thresh_zstats (3.0)	8	0.66	3.48	1.33	0,-28,2
	Low	%S.C.	--	0.11	0.35	0.11	0,-34,2
		zstats	40	1.10	2.87	1.03	0,-28,2
		thresh_zstats (2.3)	6	0.39	2.87	0.94	0,-28,2
		thresh_zstats (3.0)	0	--	--	--	--
	High>Low	%S.C.	--	0.04	0.16	0.05	0,-36,4
		zstats	40	0.52	1.77	0.59	0,-36,4
		thresh_zstats (2.3)	0	--	--	--	--

there were three significant positive FCs between the PG and the right and left thalamus as well as the vermis, whereas only one significant negative FC was observed with the left superior parietal.

The FC of the PG with brain structures, when comparing the high and low load conditions (High > Low), did not survive the multiple comparisons (by FWER); however, the uncorrected results showed a positive FC between the PG and the left postcentral gyrus and the right inferior lateral occipital and a negative FC with the precuneus.

3.4. Associations with the Memory Scores

As stated in Methods, the association of the PG volume with the memory scores was assessed as another possible evidence of the involvement of this brain area in the memory function. The associations were with the scores of the RAVLT test, Benson test, FDST, BDST, and the N-back test.

Table 3. The functional connectivity (FC) of the PG with the 27 brain structures (separately for the left and right hemispheres), in the three conditions of High, Low, and High > Low; the estimations here were solely based on the thresholded PG atlas. T(30) = t-value with 30 degrees-of-freedom; p-unc = uncorrected p-value; p-FDR = p-value corrected based on the False Discovery Rate; n.s. = non-significant; r = right; l = left; SPL = superior parietal; iLOC = inferior lateral occipital; sLOC = superior lateral occipital; SMA = supplementary motor area; CG = central gyrus

Condition	FC	Seed	T(30)	p-unc	p-FDR
High Load Condition	**Positive FC**	Thalamus r	3.67	0.0009	0.0288
		Thalamus l	3.46	0.0017	0.0288
		Vermis	3.24	0.0029	0.0376
		Caudate r	2.07	0.0471	n.s.
	Negative FC	SPL l	-3.59	0.0012	0.0288
		Precuneous	-2.98	0.0057	n.s.
		sLOC l	-2.36	0.0248	n.s.
		SMA l	-2.32	0.0271	n.s.
		sLOC r	-2.23	0.0335	n.s.
Low Load Condition	**Positive FC**	Thalamus r	4.34	0.0001	0.0039
		Thalamus l	3.58	0.0012	0.0122
		Hippocampus r	2.83	0.0083	n.s.
		Parahipp. r	2.4	0.0226	n.s.
		Hippocampus l	2.53	0.017	n.s.
	Negative FC	PostCG l	-5.19	0	0.0007
		PostCG r	-3.89	0.0005	0.0081
		SPL l	-3.82	0.0006	0.0081
		PreCG l	-3.42	0.0018	0.0158
		iLOC r	-3.23	0.003	0.0224
		SPL r	-2.68	0.0118	n.s.
		iLOC l	-2.42	0.0217	n.s.
		PreCG r	-2.14	0.0405	n.s.
High>Low Condition	**Positive FC**	PostCG l	3.49	0.0015	n.s.
		iLOC r	2.89	0.0071	n.s.
	Negative FC	Precuneous	-3.00	0.0054	n.s.

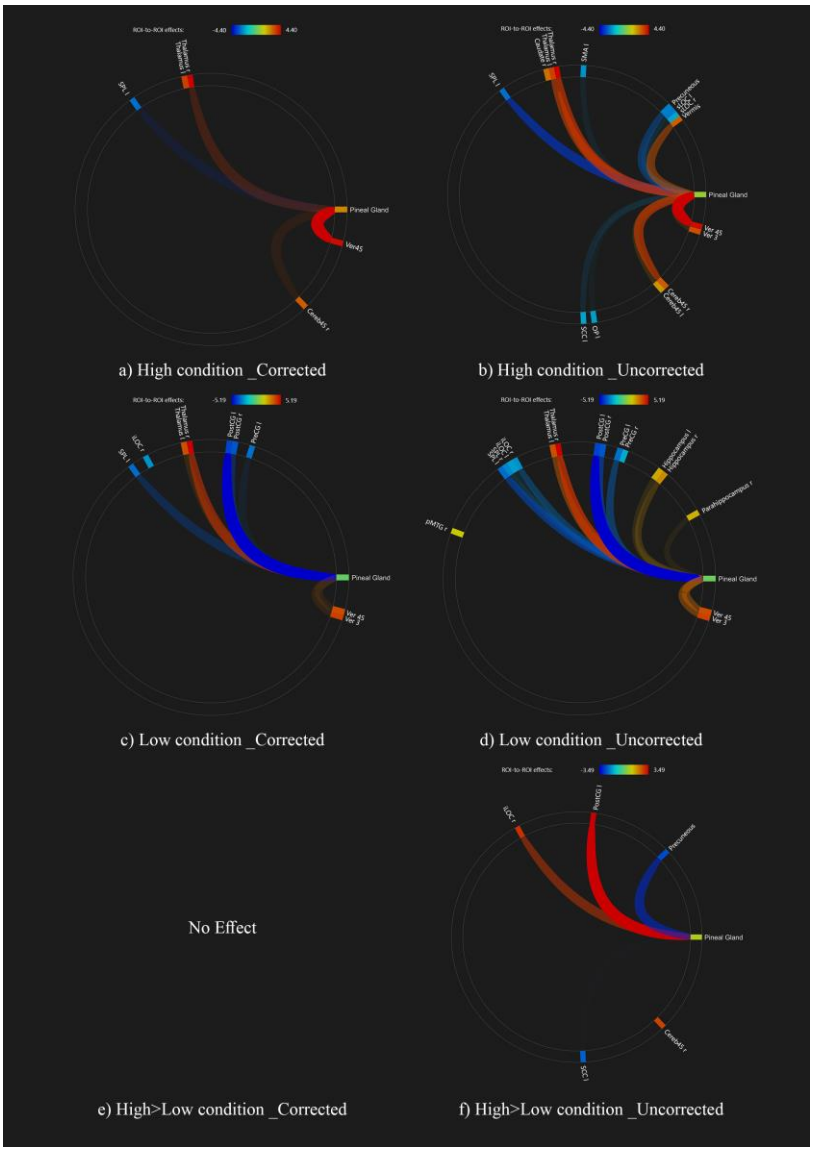

Figure 3. The functional connectivity (FC) of the PG with the 27 brain structures (separately for the left and right hemispheres), in the three conditions of High, Low, and High > Low; the estimations here were solely based on the thresholded PG atlas. Warm and cool colors represent the positive and negative FC, respectively. The thickness of the lines also represents the strength of the FC. r = right; l = left; SPL = superior parietal; iLOC = inferior lateral occipital; sLOC = superior lateral occipital; SMA = supplementary motor area; CG = central gyrus

The associations of the PG volume with the scores of the memory tests, along with the associations of 48 other brain structures, are provided in Figures 4 and 5. The results of other brain structures are provided for comparison purposes. About the PG, the volume of this area showed a significant (p-value < 0.05, uncorrected) positive association with the DR (r = 0.17), FAL (r = 0.22), TL (r = 0.24), and NPS (r = 0.26) measures of the RAVLT test and a significant (p-value < 0.05, uncorrected) negative association with the Recall Time (r = -0.15) of the Benson test. In addition, all four scores of the Forward and Backward DST tests showed significant (uncorrected p-value < 0.05) associations with the PG volume, with negative associations (uncorrected p-value < 0.05) observed in the min-time (r = -0.16) and average-time (r = -0.19) scores of the N-back test. These associations suggest that a higher PG volume results in better cognitive performance, either through a higher score on the test (positive association) or through a lower time (negative association). However, among these associations, only the correlation coefficient of the PG volume with the NPS measure of the RAVLT tests survived the multiple comparisons correction (p < 0.05, FWER corrected), whereas some other brain structures had no FWER-survived association with the cognitive measures.

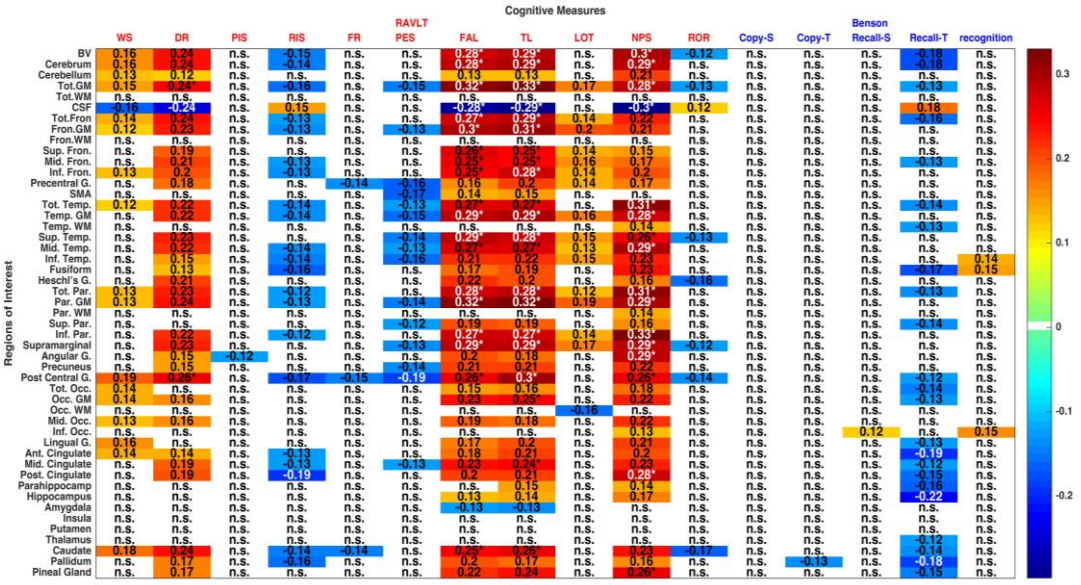

Figure 4. The association of the volumes of 48 brain structures, in addition to the PG, with the scores of the RAVLT and Benson memory tests; color cells show a p-value < 0.05 for the associations (uncorrected), and the asterisk (*) shows a significant p-value after multiple comparisons correction (FWER). n.s. = non-significant; GM = grey matter; WM = white matter; Fron = frontal; Temp = temporal; Occ = occipital; Par = parietal; G = gyrus; RAVLT scores (A1 = WS, A2 = DR, A3 = PIS, A4 = RIS, A5 = FR, A6 = PES, B1 = FAL, B2 = TL, B3 = LOT, C1 = NPS, C2 = ROR); S= score; T= time

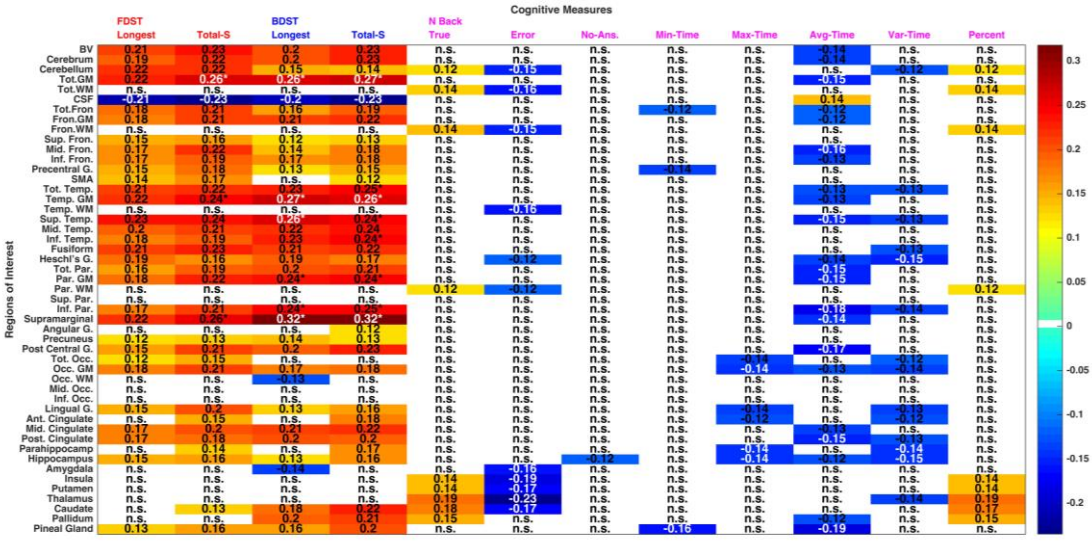

Figure 5. The association of the volumes of 48 brain structures, in addition to the PG, with the scores of the FDST and BDST tests (Forward and Backward Digit Span Tasks), as well as the one-back test; color cells show a p-value < 0.05 for the association (uncorrected), and the asterisk (*) shows a significant p-value after multiple comparisons correction (FWER). n.s.= non-significant; GM= grey matter; WM = white matter; Fron = frontal; Temp = temporal; Occ = occipital; Par = parietal; G = gyrus

4. Discussion

4.1. Summary of the Study

The PG is a less-studied brain structure due to its small size, the unavailability of its parcellation maps in the standard brain templates, as well as the general belief that this structure is solely responsible for melatonin secretion. There were recent suggestions that this brain structure may be involved in some cognitive functions such as memory, and therefore we designed a series of works in order to empirically test this idea. In the current study, we have provided some initial evidence for the involvement of the PG in the human memory system. Our claim was based on the following results: I) the PG

activation in fMRI during a memory task; II) its altered functional connectivity with other brain structures when the load of the memory retrieval changed; and III) observing the association of the PG volume with the memory abilities of the individuals. By the way, we suggest that further studies are required to test the replicability of our findings.

4.2. The Effects of Cognitive Load

Our initial finding on the involvement of the PG in the memory function was from the fMRI data in which the PG showed activations during a memory retrieval task, and its activation increased with the higher load of the retrieval. In the original study [29], seven brain structures showed a higher activation when retrieving higher load images compared to low; however, the PG was not observed active there, as we did not have access to the standard parcellation maps of this brain area [4] at that time.

The activation of those seven brain structures is evidence for their involvement in the memory function. There are similar previous findings on that; for example, the precuneus is illustrated to be involved in the storage of information [52] and visual imagery during episodic retrieval [53], and its activation was also associated with the period a memory was maintained in the brain [54]. The occipital brain areas also were active in providing visual imagery when people remembered what visual scenes looked like [55], and the cerebellum also has a role in spatial memory, working memory [56], and sensory memory [57]. As a result, we could suggest a similar interpretation for the activation of the PG during LTM retrieval. There are explanations for the brain activity increment in response to an increasing load; examples include a higher computational demand in the regions involved in that function [58], utilizing a higher-level cognitive control process in a higher load [59], or the brain regions to track task difficulty and therefore working harder to perform a more difficult task [60]. A study has suggested that if a brain area is crucial for memory, its activity should be modulated by the memory load [61], and therefore our findings may imply that the PG is involved in the memory function, as it showed an altered activation in an altered load of the LTM retrieval.

The second piece of evidence was based on the altered FC of the PG with other brain regions when the LTM load changed. Albeit, some of these results did not survive the multiple comparisons; however, the uncorrected results showed a higher FC of the PG with the right inferior lateral occipital cortex and the left postcentral gyrus in the high load compared to the low load condition, as well as a declined FC with the precuneus in the same condition. In our previous study, the left inferior temporal gyrus and the right superior lateral occipital cortex showed a stronger FC with the precuneus in the higher load, whereas the right and left angular gyri showed a declined FC with precuneus in the same condition. The brain areas that showed an altered FC were illustrated to be involved in the memory function, for example, the precuneus being involved in recollection, mental imagery strategies, cue reactivity, and episodic memory retrieval [62]. It seems one mechanism for retrieving scenes with much higher details and colors is a stronger connectivity between the brain areas that have similar functions. In a study on episodic memory encoding, an altered (increased) functional connectivity was observed between the left hippocampus and the bilateral ventrolateral prefrontal cortex and the right temporoparietal junction in a deep compared to shallow encoding [63]. Changes of the FC between the brain areas due to an altering cognitive load are also observed, such as between the fusiform, IFG, and hippocampus [64], and these load-dependent changes of FC suggest that these neural circuits dynamically trade-off to accommodate the particular demands of the task [64]. As a result, observing changes in the FC of the PG could be one other suggested evidence for its involvement in the memory function.

4.3. Associations with the Memory Scores

We observed associations between the volume of the PG and the scores of some memory tests, in particular the RAVLT test (c1 = net positive score). Based on those results, a higher PG volume was associated with a better memory ability. The volume of the PG has previously shown associations with many different parameters; examples include the head circumference, body height and body weight [6], age, such as increments from puberty to older ages [65] and decrements in the elderly [4, 66], with more insomnia reports in older adults due to the PG volume decline [19], higher consumption of coffee during the lifetime being associated with a lower PG volume which also impairs the quality of sleep in the elderly [67], as well as its associations with the volume

of other brain structures, as shown previously [4, 68]. In one study, a correlation was reported between the decline in the PG volume and the cognitive decline [21]. A similar report is also available for its neighbor structure, in which the association of the volume of the mammillary bodies with the cognitive abilities, and in particular the recollective memory function, was illustrated [69].

4.4. PG in Brain Functions

There are reports on the indirect involvement of the PG in brain functions; for example, one study found that individuals with primary insomnia have smaller pineal volumes [19], or another study identified that the decrements of this gland are positively correlated with sleep-rhythm disturbances [70]; the disruptions in the circadian rhythm and sleep/wake patterns were later considered as the indirect role of the PG in the evolution or progression of the affective and thought-related disorders [71]. One longitudinal study revealed that the PG volume is significantly lower in schizophrenia and at-risk mental disorders; the results that led the authors to speculate that a smaller PG might be a non-dynamic predisposing agent for schizophrenia [72]. There are also rare case reports of improvements in psychiatric symptoms after the disappearance of the tumors in the PG [73]. However, despite the widespread investigations, the evidence is inconclusive regarding the lower or higher PG volume and melatonin concentrations and the advance or delay in melatonin's temporal occurrence within the abnormal neuropsychiatric conditions [74]. Melatonin participation spans a plethora of fundamental physiological activities, from adjusting the chronobiological rhythms [75] and controlling the frame of the neuroplasticity-associated signaling [76], to the hypothesized modulation of sensation [77], perception [78], mood [79], and cognitive capabilities [80]. A remarkable negative correlation between the concentrations of melatonin and the rate of cognitive impairment and depressed mood was indicated in a broad elderly population so that the specific interaction between the melatonin levels and cognitive functions was unrelated to depressive symptoms. Alzheimer's disease as a pathological aging state also indicated that the volume of the PG and melatonin level strongly predicted the neurocognitive states in patients with AD [81], and another study confirmed that lower melatonin level correlates with lower-quality outputs in cognitive assessments in schizophrenia and healthy participants [82].

Although there could hardly be a clear distinction between the direct or indirect role of a brain structure in cognitive abilities, associations are more considered a direct involvement. We observed significant associations between the PG volume and the cognitive scores, and the associations between the brain structure and cognitive performance are very well established. A few examples include the reports on the association between brain atrophy and cognitive decline [83], the size of the brain and intelligence [84], and the association between brain size and mental abilities [85]. Also, it was illustrated that the decline of GM in the elderly was a reason for poorer executive functioning and processing speed [86]. In our results, total GM volume showed a significant association with our cognitive tests, with the maximum correlation coefficients of 0.33 with the TL score-RAVLT test, the pattern which was in agreement with previous works [86, 87]. The local volumetric measures also showed associations with brain cognition, and there are numerous reports of the involvement of the local brain areas in cognition, such as the inferior parietal [88], superior temporal cortex [89], caudate [90], and inferior frontal gyrus [91]. Our results showed that, similar to other brain ROIs which show an association with brain cognition, the PG also plays a role. Our literature review did not result in many reports on the cognitive involvement of the PG; one study suggested its possible role in religious meditation [26], as it showed activation during the mental operation period of silent recitation of specific religious phrases and mental imagination of receiving spiritual energy. Another study related the PG to the conscious life [92]; they defined a neuronal circuit and named it the amygdala-habenula-pineal (AHAP) functional axis, and suggested that the AHAP axis would be more connected to the conscious life, then to value-based decisions and to the freedom in the choices of our life. The fundamental role of the habenula is also justified by its relation with the PG, the only human organ able to transduce the environmental electromagnetic conditions into a modulation of the psychobiological response [93]. The relationships between the amygdala and the pineal-habenular complex would be important in determining an adequate emotional response to the different environmental and social conditions. In fact, the pineal hormone melatonin, the most investigated pineal hormone, has appeared to be a potential regulator of memory formation and other

cognitive processes through its influence on amygdala sensitivity, hippocampus-amygdala interactions, and habenula activity [93, 94]. Moreover, the PG is the main organ responsible for the production of beta-carbolines, a group of molecules provided by antitumor, antidepressant, and psychedelic properties, which would play a fundamental role in the regulation of consciousness states. The fundamental role of the PG in regulating the status of consciousness and the expansion of the mind is also confirmed by the evidence of an altered neuroendocrine pineal function in cognitive disorders, including schizophrenia, autism, and dementia [93].

We have suggested the role of the PG in the human memory function in our work, but there are other reports on that as well; example findings include melatonin being able to prevent spatial and non-spatial memory impairments [95], melatonin having the potential to diminish the negative effects of Methotrexate on memory and neurogenesis [96], melatonin therapy alleviating memory impairment through switching microglial polarization from M1 to M2 phenotype along with altered expression and function in the BDNF/TrkB/CREB signaling pathway [97], high-fat diet inducing memory impairments and the melatonin preventing this impairment probably by preventing alteration of oxidative stress in the hippocampus [98], and melatonin being effective in protecting memory deficit, oxidative stress and neuronal damage induced by streptozotocin [99]. As a result, the role of PG in the memory function could be more seriously considered, but there are still questions on the exact mechanism of this involvement and in addition on the stage of the memory formation in which the PG is involved.

5. Conclusion

In our previous study, we declared that our current knowledge of the human LTM system is not able to clearly explain this process, in particular, in terms of the location of memory storage [27, 30, 100]. Multiple neuroimaging studies have already been performed on the LTM in humans and during the retrieval of information, but none of them can distinguish between the brain regions involved in memory retrieval, memory storage, and memory processing. There are brain regions reported to be involved in LTM formation, but there is no consensus on the specific brain region(s) responsible for the storage of LTM [101]. One current suggestion is that memories are distributed throughout the brain [102, 103], but there is no strong evidence of that either. We hope our finding on the role of the PG in the human memory system could help to unravel the mechanism of LTM storage in the human brain. A study declared that understanding the material basis of memory remains a central goal of modern neuroscience, and therefore a great deal of experimental investment should be directed towards questions regarding the mechanisms of memory storage [104].

5.1. Limitations

Despite the endeavors, our study had some limitations. First, as the PG is not a neuron but a gland, the elaborate reason that causes the fMRI signal changes in PG still needs more study; albeit, there are reports on the PG having neurons. Second, this brain structure does have a small size, and the issue of tissue contamination should be very carefully controlled when analyzing its function or structure. Third, associations do not necessarily represent causality, therefore we should be cautious when suggesting a causal role for the PG in memory. And finally, our study was only a beginning to test the hypothesis and further works with different approaches are needed for a consensus; we suggest that performing animal studies could also be helpful here.

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References

1- Forooq Razavi, Samira Raminfard, Hadis Kalantar, Minoo Sisakhti, and Seyed Amir Hossein Batouli, "A probabilistic atlas of the pineal gland in the standard space." *Frontiers in Neuroinformatics*, Vol. 15p. 19, (2021).

2- Pejman Kiani, Gholamreza Hassanzadeh, Seyed Behnamedin Jameie, and Seyed Amir Hossein Batouli, "Exploration of the white matter bundles connected to the pineal gland: A DTI study." *Surgical and Radiologic Anatomy*, (2024).

3- Ingo Nölte *et al.*, "Pineal volume and circadian melatonin profile in healthy volunteers: An interdisciplinary approach." *Journal of Magnetic Resonance Imaging*, Vol. 30pp. 499-505, (2009).

4- Minoo Sisakhti, Lida Shafaghi, and Seyed Amir Hossein Batouli, "The Volumetric Changes of the Pineal Gland with Age: An Atlas-based Structural Analysis." *Experimental Aging Research,* pp. 1-31, (2022).

5- Lara G Sigurdardottir *et al.*, "Pineal Gland Volume Assessed by MRI and Its Correlation with 6-Sulfatoxymelatonin Levels among Older Men." (in eng), *Journal of biological rhythms,* Vol. 31pp. 461-69, (2016).

6- Bo Sun *et al.*, "The pineal volume: a three-dimensional volumetric study in healthy young adults using 3.0T MR data." *International Journal of Developmental Neuroscience,* Vol. 27pp. 655-60, (2009).

7- Akio Hasegawa, Kohichiro Ohtsubo, and Wataru Mori, "Pineal gland in old age; quantitative and qualitative morphological study of 168 human autopsy cases." *Brain Research,* Vol. 409pp. 343-49, (1987).

8- M S Raghuprasad and M Manivannan, "Volumetric and Morphometric Analysis of Pineal and Pituitary Glands of an Indian Inedial Subject." (in eng), *Annals of neurosciences,* Vol. 25pp. 279-88, (2018).

9- Wajd N Al-Holou, Cormac O Maher, Karin M Muraszko, and Hugh J L Garton, "The natural history of pineal cysts in children and young adults." (in English), *Journal of Neurosurgery: Pediatrics PED,* Vol. 5pp. 162-66, (2010).

10- R E Silman., R M Leone, R J L Hooper, and M A Preece, "Melatonin, the pineal gland and human puberty." *Nature,* Vol. 282pp. 301-03, (1979).

11- B Goldman *et al.*, "Chronobiology: biological timekeeping." *Circannual rhythms and photoperiodism,* pp. 107-42, (2004).

12- C Cajochen, K Kräuchi, and A Wirz-Justice, "Role of Melatonin in the Regulation of Human Circadian Rhythms and Sleep." *Journal of Neuroendocrinology,* Vol. 15pp. 432-37, (2003).

13- Janusz Golan, Kamil Torres, Grzegorz Staśkiewicz, Grzegorz Opielak, and Ryszard Maciejewski, "Morphometric parameters of the human pineal gland in relation to age, body weight and height." *Folia morphologica,* Vol. 61pp. 111-13, (2002).

14- P. F. Reyes, "Age related histologic changes in the human pineal gland." *Progress in clinical and biological research,* Vol. 92pp. 253-61, (1982).

15- Russel Reiter *et al.*, "Light at Night, Chronodisruption, Melatonin Suppression, and Cancer Risk: A Review." *Critical reviews in oncogenesis,* Vol. 13pp. 303-28, (2007).

16- Samuel Sarrazin *et al.*, "MRI exploration of pineal volume in bipolar disorder." *Journal of Affective Disorders,* Vol. 135pp. 377-79, (2011).

17- Shigeru Nishida, "Metabolic effects of melatonin on odative stress and dbetes mellitus." *Endocrine,* Vol. 27pp. 131-35, (2005).

18- D Larry Sparks and John C Hunsaker III, "The Pineal Gland in Sudden Infant Death Syndrome: Preliminary Observations." *Journal of Pineal Research,* Vol. 5pp. 111-18, (1988).

19- Jan M Bumb *et al.*, "Pineal gland volume in primary insomnia and healthy controls: a magnetic resonance imaging study." *Journal of Sleep Research,* Vol. 23pp. 276-82, (2014).

20- Ebru Fındıklı, Mehmet Inci, Mustafa Gökçe, Hüseyin Fındıklı, Hatice Altun, and Mehmet Karaaslan, "Pineal gland volume in schizophrenia and mood disorders." *Psychiatria Danubina,* Vol. 27pp. 153-58, (2015).

21- Teruyuki Matsuoka *et al.*, "Reduced Pineal Volume in Alzheimer Disease: A Retrospective Cross-sectional MR Imaging Study." *Radiology,* Vol. 286p. 170188, (2017).

22- Martin Grosshans *et al.*, "The association of pineal gland volume and body mass in obese and normal weight individuals: A pilot study." *Psychiatria Danubina,* Vol. 28pp. 220-24, (2016).

23- Murad Atmaca, Tuba Korucu, M Caglar Kilic, Asli Kazgan, and Hanefi Yildirim, "Pineal gland volumes are changed in patients with obsessive-compulsive personality disorder." *Journal of Clinical Neuroscience,* Vol. 70pp. 221-25, (2019).

24- Richard Mahlberg, Thorsten Kienast, Sven Hädel, Jens Olaf Heidenreich, Stephan Schmitz, and Dieter Kunz, "Degree of pineal calcification (DOC) is associated with polysomnographic sleep measures in primary insomnia patients." *Sleep Medicine,* Vol. 10pp. 439-45, (2009).

25- F. López-Muñoz, J. D. Molina, G. Rubio, and C. Alamo, "An historical view of the pineal gland and mental disorders." *Journal of Clinical Neuroscience,* Vol. 18pp. 1028-37, (2011).

26- Chien-Hui Liou, Changwei Hsieh, Chao-Hsien Hsieh, Si-chen Lee, J Chen, and Chi-Hong Wang, "Correlation between Pineal Activation and Religious Meditation Observed by Functional Magnetic Resonance Imaging." *Nature Precedings,* Vol. 2(2007).

27- Seyed Amir Hossein Batouli and Minoo Sisakhti, "Investigating A Hypothesis on The Mechanism of Long-Term Memory Storage." *NeuroQuantology,* Vol. 17(2019).

28- Seyed Amir Hossein Batouli *et al.*, "Iranian Brain Imaging Database: A Neuropsychiatric Database of Healthy Brain." *BCN,* Vol. 12pp. 115-32, (2021).

29- Minoo Sisakhti, Perminder S Sachdev, and Seyed Amir Hossein Batouli, "The Effect of Cognitive Load on the Retrieval of Long-Term Memory: An fMRI Study " in Frontiers in Human Neuroscience Vol. 15 ed, (2021), p. 606.

30- Seyed Amir Hossein Batouli, "Seven Ambiguities in Explaining the Human Memory System in the Principles of Neural Science Book." *BCN,* Vol. 14 (No. 4), pp. 543-48, (2023).

31- Seralynne D Vann and Andrew J D Nelson, "Chapter 9 - The mammillary bodies and memory: more than a hippocampal relay." in The Connected Hippocampus Vol. 219, Shane O'Mara and Marian B T - Progress in Brain Research Tsanov, Eds., ed: Elsevier, (2015), pp. 163-85.

32- Okihide Hikosaka, "The habenula: from stress evasion to value-based decision-making." *Nature Reviews Neuroscience,* Vol. 11pp. 503-13, (2010).

33- D Sanders *et al.*, "Nicotinic receptors in the habenula: importance for memory." *Neuroscience,* Vol. 166pp. 386-90, (2010).

34- Lucas Lecourtier, Hans C Neijt, and Peter H Kelly, "Habenula lesions cause impaired cognitive performance in rats: implications for schizophrenia." *European Journal of Neuroscience,* Vol. 19pp. 2551-60, (2004).

35- Mayur Sharma, Venkatesh Madhugiri, and Anil Nanda, "James L. Poppen and Surgery of the "Seat of the Soul": A Contemporary Perspective." *World Neurosurgery,* Vol. 82pp. 529-34, (2014).

36- Seyed Amir Hossein Batouli and Minoo Sisakhti, "Some Points to Consider in a Task-Based fMRI Study: A Guideline for Beginners." *Frontiers in Biomedical Technologies,* Vol. 7(2020).

37- Julie D Henry and John R Crawford, "The short-form version of the Depression Anxiety Stress Scales (DASS-21): Construct validity and normative data in a large non-clinical sample." *British journal of clinical psychology,* Vol. 44pp. 227-39, (2005).

38- Ali Sahebi, Mohammad Javad Asghari, and Razie Sadat Salari, "Validation of depression anxiety and stress scale (DASS-21) for an Iranian population." *Iranian Psychologists,* Vol. 4pp. 299-313, (2005).

39- Maura Mitrushina, Paul Satz, Alexander Chervinsky, and Lou D'Elia, "Performance of four age groups of normal elderly on the Rey Auditory-Verbal Learning Test." *Journal of Clinical Psychology,* Vol. 47pp. 351-57, (1991).

40- Minoo Sisakhti, Seyed Amir Hossein Batouli, and Hassan Farrahi, "The Rey Auditory Verbal Learning Test: Age-, Gender- and Education-Related Normative Data for The Iranian Healthy Population." *Frontiers in Biomedical Technologies,* Vol. 10 (No. 3), 06/01 (2023).

41- Aline Ferreira Correia and Ilva Campagna Osorio, "The Rey auditory verbal learning test: normative data developed for the venezuelan population." *Archives of Clinical Neuropsychology,* Vol. 29pp. 206-15, (2013).

42- Mehrnaz Rezvanfard, Hamed Ekhtiari, and Maryam Noroozian, "The Rey Auditory Verbal Learning Test: alternate forms equivalency and reliability for the Iranian adult population (Persian version)." *Archives of Iranian Medicine,* Vol. 14p. 104, (2011).

43- Katherine L Possin, Victor R Laluz, Oscar Z Alcantar, Bruce L Miller, and Joel H Kramer, "Distinct neuroanatomical substrates and cognitive mechanisms of figure copy performance in Alzheimer's disease and behavioral variant frontotemporal dementia." *Neuropsychologia,* Vol. 49pp. 43-48, (2011).

44- Minoo Sisakhti, Helia Hosseini, Seyed Amir Hossein Batouli, and Hassan Farrahi, "The Benson Complex Figure Test: Normative Data for the Healthy Iranian Population." *Frontiers in Biomedical Technologies,* Vol. 11(2024).

45- Mónica Rosselli *et al.*, "Effects of Bilingualism on Verbal and Nonverbal Memory Measures in Mild Cognitive Impairment." *Journal of the International Neuropsychological Society,* Vol. 25pp. 15-28, (2019).

46- David Wechsler, "Wechsler memory scale-revised." *Psychological Corporation,* (1987).

47- Minoo Sisakhti, Seyed Amir Hossein Batouli, Elaheh Delazar, and Hassan Farrahi, "The Digit Span Test: Normative Data for the Iranian Normal Population TT - ." *gums-cjns,* Vol. 10pp. 182-93, (2024).

48- Robert L Hester, Glynda J Kinsella, and B E N Ong, "Effect of age on forward and backward span tasks." *Journal of the International Neuropsychological Society,* Vol. 10pp. 475-81, (2004).

49- Michael J Kane, Andrew R A Conway, Timothy K Miura, and Gregory J H Colflesh, "Working memory, attention control, and the N-back task: a question of construct validity." *Journal of Experimental Psychology: Learning, Memory, and Cognition,* Vol. 33p. 615, (2007).

50- MahinNaz Mirdehghan, Vahid Nejati, and Golnaz Ganjian, "Working Memory in regard to Persian and Chinese words for Persian Learners of Chinese." *mdrsjrns,* Vol. 7pp. 197-213, (2016).

51- Gaurav Vivek Bhalerao *et al.*, "Construction of population-specific Indian MRI brain template: Morphometric comparison with Chinese and Caucasian templates." *Asian Journal of Psychiatry,* Vol. 35pp. 93-100, (2018).

52- Alan A Hartley and Nicole K Speer, "Locating and fractionating working memory using functional neuroimaging: Storage, maintenance, and executive functions." *Microscopy Research and Technique,* Vol. 51pp. 45-53, (2000).

53- Andrea E Cavanna and Michael R Trimble, "The precuneus: a review of its functional anatomy and behavioural correlates." *Brain,* Vol. 129pp. 564-83, (2006).

54- Sander M Daselaar, Heather J Rice, Daniel L Greenberg, Roberto Cabeza, Kevin S LaBar, and David C Rubin, "The Spatiotemporal Dynamics of Autobiographical Memory: Neural Correlates of Recall, Emotional Intensity, and Reliving." *Cerebral Cortex* Vol. 18 pp. 217-29, (2008).

55- Noa Raz and Netta Levin, "Cortical and white matter mapping in the visual system-more than meets the eye: on the importance of functional imaging to understand visual

system pathologies " in Frontiers in Integrative Neuroscience Vol. 8 ed, (2014), p. 68.

56- Matthew P Kirschen, S H Annabel Chen, Pamela Schraedley-Desmond, and John E Desmond, "Load- and practice-dependent increases in cerebro-cerebellar activation in verbal working memory: an fMRI study." *NeuroImage,* Vol. 24pp. 462-72, (2005).

57- Augusto Petacchi, Angela R Laird, Peter T Fox, and James M Bower, "Cerebellum and auditory function: An ALE meta-analysis of functional neuroimaging studies." *Human Brain Mapping,* Vol. 25pp. 118-28, (2005).

58- Ada W S Leung and Claude Alain, "Working memory load modulates the auditory "What" and "Where" neural networks." *NeuroImage,* Vol. 55pp. 1260-69, (2011).

59- David Fegen, Bradley R Buchsbaum, and Mark D'Esposito, "The effect of rehearsal rate and memory load on verbal working memory." *NeuroImage,* Vol. 105pp. 120-31, (2015).

60- R L Gould, R G Brown, A M Owen, D H ffytche, and R J Howard, "FMRI BOLD response to increasing task difficulty during successful paired associates learning." *NeuroImage,* Vol. 20pp. 1006-19, (2003).

61- Hoi-Chung Leung, David Seelig, and John C Gore, "The effect of memory load on cortical activity in the spatial working memory circuit." *Cognitive, affective & behavioral neuroscience,* Vol. 4pp. 553-63, (2005).

62- David Borsook, Nasim Maleki, and Rami Burstein, "Chapter 42 - Migraine." Michael J Zigmond, Lewis P Rowland, and Joseph T B T - Neurobiology of Brain Disorders Coyle, Eds., ed. San Diego: Academic Press, (2015), pp. 693-708.

63- Björn Schott *et al.*, "The relationship between level of processing and hippocampal-cortical functional connectivity during episodic memory formation in humans." *Human Brain Mapping,* Vol. 34(2013).

64- Jesse Rissman, Adam Gazzaley, and Mark D'Esposito, "Dynamic adjustments in prefrontal, hippocampal, and inferior temporal interactions with increasing visual working memory load." (in eng), *Cerebral cortex (New York, N.Y. : 1991),* Vol. 18pp. 1618-29, (2008).

65- E Tapp and Marianne Huxley, "The histological appearance of the human pineal gland from puberty to old age." *The Journal of Pathology,* Vol. 108pp. 137-44, (1972).

66- Jan M Bumb, Marc A Brockmann, Christoph Groden, and Ingo Nolte, "Microstructural analysis of pineal volume using trueFISP imaging." (in eng), *World journal of radiology,* Vol. 5pp. 166-72, (2013).

67- Jeongbin Park *et al.*, "Lifetime coffee consumption, pineal gland volume, and sleep quality in late life." *Sleep,* Vol. 41(2018).

68- Julius Axelrod, Richard J Wurtman, and Solomon H Snyder, "Control of Hydroxyindole O-Methyltransferase Activity in the Rat Pineal Gland by Environmental Lighting." *Journal of Biological Chemistry* Vol. 240 pp. 949-54, (1965).

69- Dimitris Tsivilis *et al.*, "A disproportionate role for the fornix and mammillary bodies in recall versus recognition memory." *Nature Neuroscience,* Vol. 11pp. 834-42, (2008).

70- Luisa Liebrich, Michael Schredl, Peter Findeisen, Christoph Groden, Jan Bumb, and Ingo Nölte, "Morphology and Function: MR Pineal Volume and Melatonin Level in Human Saliva Are Correlated." *Journal of magnetic resonance imaging : JMRI,* Vol. 40(2014).

71- Ilia Karatsoreos, "Links between Circadian Rhythms and Psychiatric Disease." *Frontiers in Behavioral Neuroscience,* Vol. 8p. 162, (2014).

72- Tsutomu Takahashi *et al.*, "Reduced pineal gland volume across the stages of schizophrenia." *Schizophrenia Research,* Vol. 206pp. 163-70, (2019).

73- Alessandro De Nadai, Eric Storch, and Jeffrey Alvaro, "Development of Obsessive-Compulsive Disorder Following a Pineal Germinoma: A Case Report." *The American journal of psychiatry,* Vol. 168pp. 550; author reply 50-1, (2011).

74- Karen Hallam, James Olver, Vanessa Chambers, Denovan Begg, Caroline McGrath, and Trevor Norman, "The heritability of melatonin sensitivity to bright nocturnal light in twins." *Psychoneuroendocrinology,* Vol. 31pp. 867-75, (2006).

75- Reed Stein *et al.*, "Virtual discovery of melatonin receptor ligands to modulate circadian rhythms." *Nature,* Vol. 579pp. 1-8, (2020).

76- leila hosseini, Fatemeh Farokhi-Sisakht, Reza Badalzadeh, Aytak Khabbaz, Javad Mahmoudi, and Saeed Sadigh-Eteghad, "Nicotinamide Mononucleotide and Melatonin Alleviate Aging-induced Cognitive Impairment via Modulation of Mitochondrial Function and Apoptosis in the Prefrontal Cortex and Hippocampus." *Neuroscience,* Vol. 423(2019).

77- T Y C Lee and Justin Curtin, "The effects of melatonin prophylaxis on sensory recovery and postoperative pain following orthognathic surgery: a triple-blind randomized controlled trial and biochemical analysis." *International Journal of Oral and Maxillofacial Surgery,* Vol. 49(2019).

78- S Aubin, Ron Kupers, Maurice Ptito, and P Jennum, "Melatonin and cortisol profiles in the absence of light perception." *Behavioural Brain Research,* Vol. 317(2017).

79- Neera Ghaziuddin *et al.*, "Salivary Melatonin Onset in Youth at Familial Risk for Bipolar Disorder." *Psychiatry Research,* Vol. 274(2019).

80- A Yun, Kimberly Bazar, and Patrick Lee, "Pineal attrition, loss of cognitive plasticity, and onset of puberty

during the teen years: Is it a modern maladaptation exposed by evolutionary displacement?" *Medical Hypotheses,* Vol. 63pp. 939-50, (2004).

81- Ying-Hui Wu and Dick Swaab, "The human pineal gland and melatonin in aging and Alzheimer's disease." *Journal of Pineal Research,* Vol. 38pp. 145-52, (2005).

82- Cigdem Sahbaz, Omer Özer, Ayse Kurtulmus, Ismet kırpınar, Fikrettin Sahin, and Sinan Guloksuz, "Evidence for an association of serum melatonin concentrations with recognition and circadian preferences in patients with schizophrenia." *Metabolic Brain Disease,* Vol. 34(2019).

83- Jasper Sluimer *et al.*, "Whole-Brain Atrophy Rate and Cognitive Decline: Longitudinal MR Study of Memory Clinic Patients 1." *Radiology,* Vol. 248pp. 590-98, (2008).

84- Michael Mcdaniel, "Big-brained people are smarter: A meta-analysis of the relationship between in vivo brain volume and intelligence." *Intelligence,* Vol. 33pp. 337-46, (2005).

85- J Rushton and C Ankney, "Whole Brain Size and General Mental Ability: A Review." *The International journal of neuroscience,* Vol. 119pp. 691-731, (2009).

86- Stephen Ramanoel *et al.*, "Gray Matter Volume and Cognitive Performance During Normal Aging. A Voxel-Based Morphometry Study." *Frontiers in Aging Neuroscience,* Vol. 10(2018).

87- Stephen Rao *et al.*, "Correlations between MRI and Information Processing Speed in MS: A Meta-Analysis." *Multiple sclerosis international,* Vol. 2014p. 975803, (2014).

88- Sarah MacPherson *et al.*, "Processing speed and the relationship between Trail Making Test-B performance, cortical thinning and white matter microstructure in older adults." *Cortex,* Vol. 95(2017).

89- Hans-Otto Karnath, "New insights into the functions of the superior temporal cortex." *Nature Reviews Neuroscience,* Vol. 2pp. 568-76, (2001).

90- Hans-Otto Karnath, "The subcortical anatomy of human spatial neglect: putamen, caudate nucleus and pulvinar." *Brain,* Vol. 125pp. 350-60, (2002).

91- Hikaru Takeuchi *et al.*, "Regional gray and white matter volume associated with Stroop interference: Evidence from voxel-based morphometry." *NeuroImage,* Vol. 59pp. 2899-907, (2012).

92- Paolo Lissoni *et al.*, "A review on the cognitive functions of basal ganglia, amygdala, hippocampus, habenula, nucleus accumbens, cerebellum, and pineal gland." *Mental Health and Addiction Research,* Vol. 5(2020).

93- Amnon Brzezinski, "Melatonin in Humans." *New England Journal of Medicine,* Vol. 336pp. 186-95, (1997).

94- Petr Bob and Peter Fedor-Freybergh, "Melatonin, consciousness, and traumatic stress." *Journal of Pineal Research,* Vol. 44pp. 341-47, (2008).

95- Anusara Aranarochana, Pornthip Chaisawang, Apiwat Sirichoat, Wanassanun Pannangrong, Peter Wigmore, and Jariya Umka Welbat, "Protective effects of melatonin against valproic acid-induced memory impairments and reductions in adult rat hippocampal neurogenesis." *Neuroscience,* Vol. 406pp. 580-93, (2019).

96- Apiwat Sirichoat *et al.*, "Melatonin protects against methotrexate-induced memory deficit and hippocampal neurogenesis impairment in a rat model." *Biochemical Pharmacology,* Vol. 163pp. 225-33, (2019).

97- Sayna Bagheri *et al.*, "Melatonin improves learning and memory of mice with chronic social isolation stress via an interaction between microglia polarization and BDNF/TrkB/CREB signaling pathway." *European Journal of Pharmacology,* Vol. 908p. 174358, (2021).

98- Karem H Alzoubi, Fadia A Mayyas, Rania Mahafzah, and Omar F Khabour, "Melatonin prevents memory impairment induced by high-fat diet: Role of oxidative stress." *Behavioural Brain Research,* Vol. 336pp. 93-98, (2018).

99- Gunjan Saxena, Sachi Bharti, Pradeep Kumar Kamat, Sharad Sharma, and Chandishwar Nath, "Melatonin alleviates memory deficits and neuronal degeneration induced by intracerebroventricular administration of streptozotocin in rats." *Pharmacology Biochemistry and Behavior,* Vol. 94pp. 397-403, (2010).

100- Seyed Amir Hossein Batouli, "Seven Ambiguities in Explaining the Human Memory System in the Principles of Neural Science Book." *Preprints,* Vol. 2021040060(2021).

101- Timothy J Teyler and Jerry W Rudy, "The hippocampal indexing theory and episodic memory: Updating the index." *Hippocampus,* Vol. 17pp. 1158-69, (2007).

102- Emanuela Santini, Thu N. Huynh, and Eric Klann, "Mechanisms of translation control underlying long-lasting synaptic plasticity and the consolidation of long-term memory." *Progress in Molecular Biology and Translational Science,* Vol. 122pp. 131-67, (2014).

103- Bennett L. Schwartz, "Memory and the Brain." in FOUNDATIONS AND APPLICATIONS, ed: SAGE Publications Inc., (2014), pp. 29-58.

104- Susumu Tonegawa, Michele Pignatelli, Dheeraj S Roy, and J Ryan, "Memory engram storage and retrieval." *Current Opinion in Neurobiology,* Vol. 35pp. 101-09, (2015).