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The Raphe-Hippocampal Tract and its Age Differences: Diffusion Tensor Imaging and Probabilistic Tractography Study

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THE RAPHE-HIPPOCAMPAL TRACT AND ITS AGE DIFFERENCES: DIFFUSION TENSOR IMAGING AND PROBABILISTIC TRACTOGRAPHY STUDY

by

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A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

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Reader: Professor John Samonds

DEDICATION

To my family who has seen me through each step of life and especially each step of this thesis. I could not have completed this without your support. Thank you for loving me and motivating me though all of the challenges, triumphs, and tribulations. It is so exciting that this is complete, and I could not have done it alone. I love you all!

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ABSTRACT

The raphe-hippocampal tract links the raphe nuclei to the hippocampus and is responsible for the production of the neurotransmitter serotonin. The hippocampus is key in regulating emotional and stress responses. This study utilized diffusion tensor imaging which uses Functional Magnetic Resonance Imaging to provide scans of the brain for analyzing differences in the raphe-hippocampal tract as one ages. In this specific study, 491 samples were visually analyzed to gather data about the fractional anisotropy of the raphe nuclei in both male and female brains ranging from 6 to 85 years old. Through the ranking of images, some were discarded, and all were evaluated based on the raphe-hippocampal tract highlighted in red and blue on the images. After analysis, the data allowed for a significant regression equation to be found. Thus, the predicted FA of the DRN-hippocampal tract is equal to 0.023 + 0.00046 (age) – 3.84 (sex). This means that as one increases in age, FA increases in the raphe-hippocampal tract. This is due to the fact that the brain shows decreasing connectivity in more external structures, creating an imbalance leading to decreased mood.

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INTRODUCTION

The raphe nucleus is located within the brainstem through the midbrain and the pons. It is classified as a nucleus that controls multiple functions. This portion of the brainstem is found to be associated with disorders involving mood such as depression and Alzheimers (Michelsen, Kimmo A, et al). The reason behind this is the fact that it is the most abundant transmitter for serotonin, a neurotransmitter that plays a key role in memory and emotion.

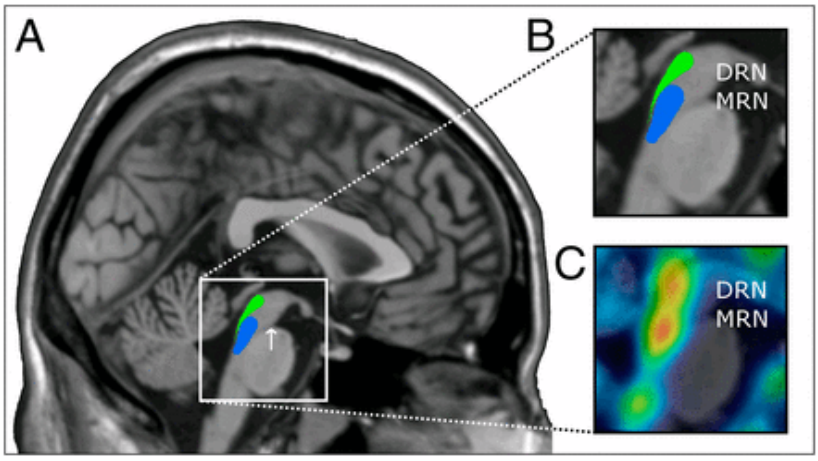

Figure 1: This figure shows the raphe nuclei. The DRN is the dorsal raphe nucleus. The MRN is the median raphe nucleus (Kranz, et al).

The structure of the raphe nucleus projects through the hippocampus carrying serotonin throughout the central nucleus, as seen in Figure 1. However, it is not isolated. The structure of nuclei receives its name because the nuclei are clustered around the midline of the brainstem. The raphe nucleus is sectioned into different parts. There is the

dorsal raphe nucleus and the median raphe nucleus (Kranz et al). The DRN contains the majority of the serotonergic neurons and will be the focus of this study.

Throughout the raphe nuclei are receptors that function as autoreceptors within the brain. Autoreceptors can be found within the membranes of presynaptic nerve cells. They are selective to specific neurotransmitters released by the neuron. These play a large part in signal transduction and hold an active role in integrating a negative feedback loop (Autoreceptor). A negative feedback loop occurs when there is an overaccumulation of substance, and in this case, of neurotransmitters. The receptor then works to reduce the output of the neurotransmitter. The name of the group of auto receptors in the raphe nucleus is 5-HT1. This name comes from the molecular name for serotonin which is 5-hydroxytryptamine. The 5-HT1 receptors play a key role in the transmission on the neurotransmitter serotonin (Quentin, et al).

Serotonin, oftentimes known as the "happy chemical," is a neurotransmitter that messages neurologic information. A neurotransmitter is a chemical substance that is diffused via the release of nerve impulses that cause a diffusion across the neural synapse, spurring the impulse onto the next neuron (Berry). Specifically, serotonin plays a large role in mood, memory, sleep patterns, and other important functions. It is incapable of crossing the blood-brain barrier. Therefore, any serotonin used within the brain must be produced in the brain, and more specifically, in the raphe nuclei (McIntosh).

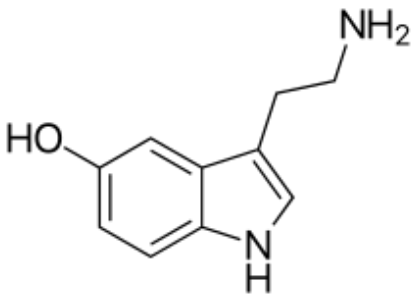

Figure 2: Molecular structure of serotonin the neurotransmitter that travels through the raphe nucleus ("Serotonin").

Serotonin is created within the raphe nucleus of the brain stem in the central nervous system. It is synthesized from the amino acid tryptophan. This occurs through a two-step process. In the first step, the enzyme tryptophan hydroxylase converts L-tryptophan into L-5OH-tryptophan. An amino acid decarboxylase then converts L-5OH-tryptophan into serotonin, so it can now act as a neurotransmitter ("Serotonin." Serotonin - an Overview). After it is made, serotonin ascends throughout several parts of the brain as shown in Figure 3. It can be seen that serotonin is made on the very inmost part of the brains and transmits outwards.

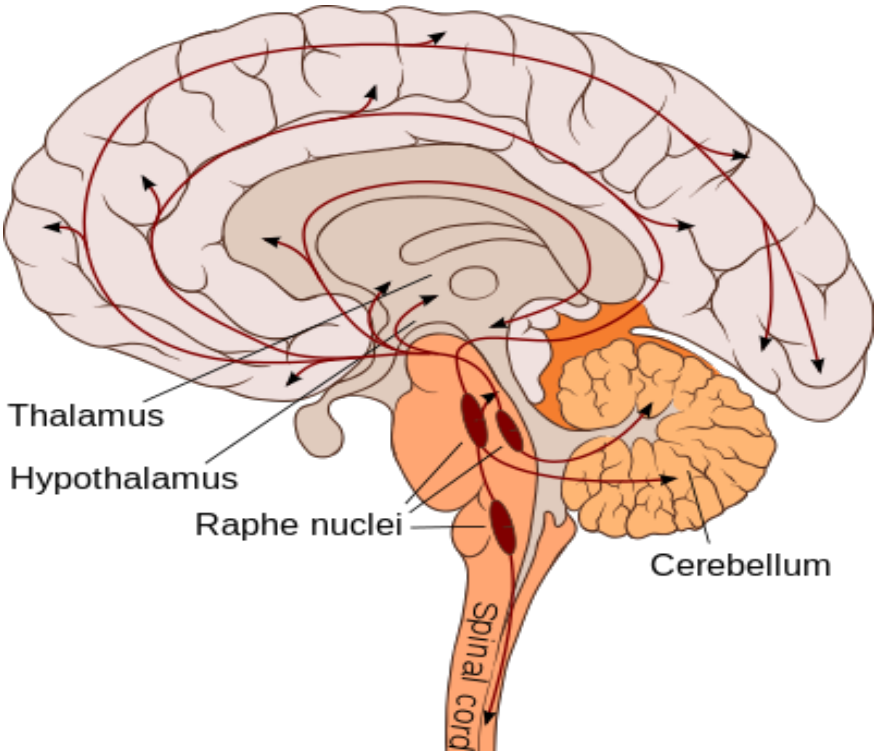

Figure 3: The pathway of the neurotransmitter serotonin after it leaves the raphe nuclei where it was made ("Serotonin Pathway").

The hippocampus is located beneath the cerebral cortex in the allocortex. This curved structure within the brain plays a large role in both formation of memory as well as emotions. It is responsible for the conversion of short-term memory into long-term memory (Boundless). The tract that is formed between the hippocampus and the raphe nucleus is found along a serotonergic projection from the median raphe nucleus to the ventral hippocampus.

Within the brain there are two different kinds of matter: grey and white. Grey matter is composed of cell bodies, dendrites, and axon terminals. This is where the synapses occur. White matter is composed of nerve fibers called axons. These axons are covered in myelin, giving the matter its white color (*GREY AND WHITE MATTER*).

In order to study the index of myelin and axon strength, fractional anisotropy is employed. Fractional anisotropy, or FA, is measured by the movement of water molecules. Isotropic movement alludes to a value of 0 while anisotropic movement would allow for a value of 1 (“Fractional Anisotropy”). Thus, this movement of water molecules can be translated into meaning how active neurons are within a structure.

METHODS

Imaging Data

DTI data were gathered from the Nathan Kline Institute-Rockland Sample (NKI-RS: http://fcon_1000.projects.nitrc.org/indi/enhanced/) (Nooner et al. 2012). NKI-RS utilizes an open neuroscience model, providing a large neuroimaging dataset with broad and deep phenotypic measures. The participants were recruited from Rockland County, NY, and composed of demographics that evenly represent the United States. Participants were screened for neurological, psychiatric, and chronic medical illnesses. There are 491 samples (42.13±20.95 range 6 to 85 years old, 180 males and 311 females) that contained DTI and WASI full scale IQ and verbal IQ data. This study contains analyses that were approved by the Institutional Review Board of the University of Mississippi (14x-244).

The DTI series had 128 volumes of noncolinear directions along with 9 volumes without diffusion weighting (TR = 2400ms, TE = 85ms, matrix = 128 × 128, FOV = 256 mm). Each of the volumes consisted of 64 contiguous 2-mm slices with 2mm^3 isotropic resolution.

Imaging Data Analysis

Through the use of the Functional Magnetic Resonance Imaging of the Brain Software Library, imaging processing took place (FSL version 4.1.8; Oxford, United Kingdom; http://fsl.fmrib.ox.ac.uk/fsl). Eddy-current induced distortions and head-motion displacements were corrected through affine registration of the 128 diffusion

volumes (b[Symbol]1500) to the first b0 volume using FSL's Linear Registration Tool. For each of the participants, the b-vector table (i.e., gradient directions) was corrected according to the rotation parameters of this linear alteration. Non-brain tissue was displaced using FSL's Brain Extraction Tool. Fractional anisotropy (FA), an index measuring the value of white matter integrity, was calculated at each voxel of the brain by fixing a diffusion tensor model to the raw diffusion data. This was done also with using weighted least squares in FSL's Diffusion Toolbox.

The local (i.e., within-voxel) probability density functions of the principal diffusion direction were approximated using Markov Chain Monte Carlo sampling in FSL's Bedpostx tool (Behrens et al. 2007). A spatial probability density function across voxels was then estimated based on these local probability density functions using FSL's Probtrackx tool (Behrens et al. 2007), in which 5000 samples were taken for each input voxel with a 0.2 curvature threshold, 0.5 mm step length, and 2000 steps per sample. Segmentation of the arcuate fasciculus was determined based on the MNI152 T1 brain provided in FSL, using FSL's FMRIB58_FA template as a DTI specific reference.

Tractography Analysis

The probabilistic tractography was conducted between the dorsal raphe nucleus and hippocampus. The dorsal raphe nucleus was defined using Harvard Ascending Arousal network Atlas in MNI152-1mm space (Edlow). The hippocampi were determined by Harvard-Oxford Subcortical Atlas (Desikan et al.). The region of interest was limited to the inferior portion of the brain (MNI Z<80). These seed ROIs and regions of interest were linearly registered to the native diffusion space of each acquisition. The

bilateral DRN-hippocampal tract of each subject was given a threshold at a normalized probability value of 0.06.

Once the images had been compiled into a folder, they needed to be evaluated. Tractography outputs for each of the 491 individuals were visually examined using in-house script. This opens images by *fslview,* allowing for a comparison of the average tract (Figure 4). The imaging revealed two distinct regions on either side of the brain which distinguished the raphe nuclei. Red was the right side and blue was the left side as seen in Figure 4. The images were scanned to see if the regions were distinct enough to use for the evaluation. An image could receive a rating from 1 being the worst to 5 being the best. Once the data was analyzed, ranked, and sometimes left with comments, it was used to create a scatterplot with a trendline. Images that were rated 2 and below were disqualified. Images that were rated as a 3 were re-examined by the second evaluator. All other images above a 3 were automatically included in the study. The mean FA within the bilateral DRM-hippocampal tract was calculated for each individual. A multiple linear regression was calculated to predict the FA of the DRN-hippocampal tract based on age.

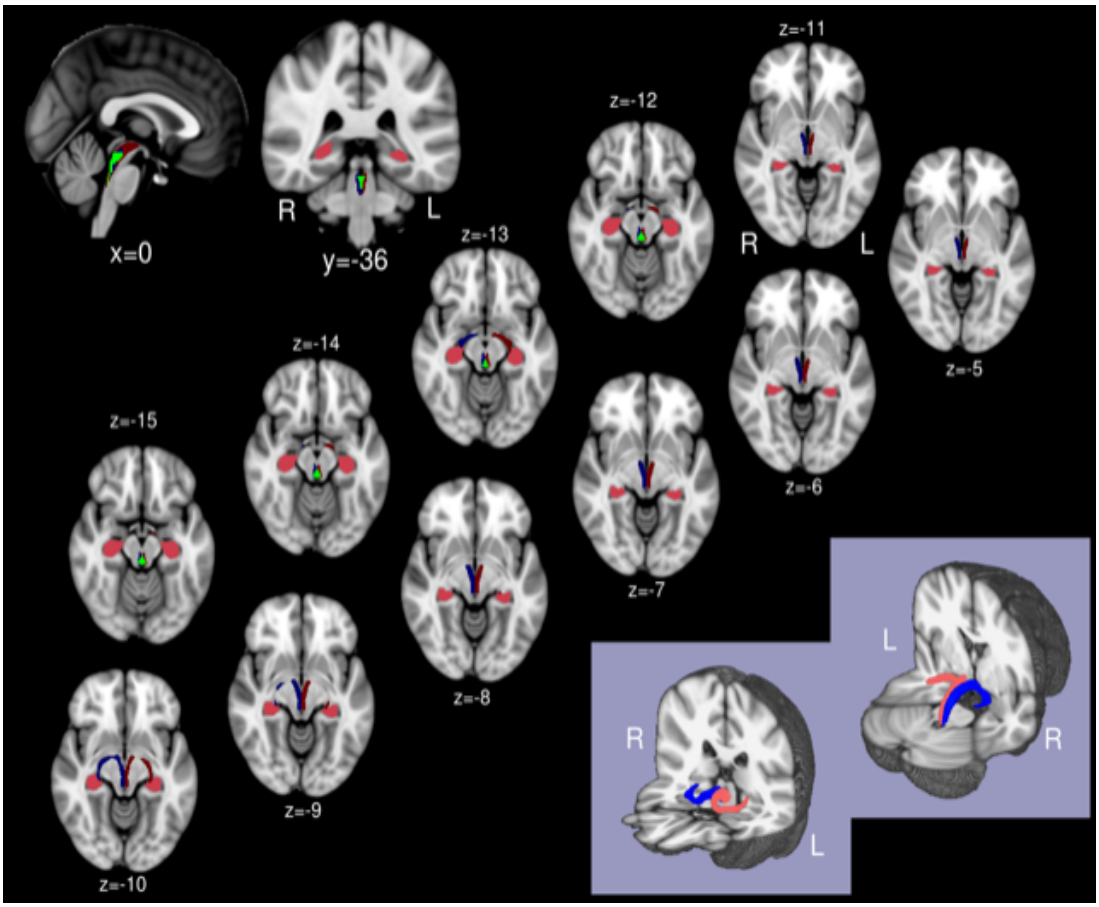

Figure 4: This figure represents the diffusion tensor imaging through Functional Magnetic Resonance Imaging. In the bottom of the figure, the raphe-hippocampal tract is highlighted in both red and blue. Red represents the left side while blue represents the right side.

RESULTS

In 461 subjects (41.68 ± 20.72 between 6 to 85 years old, 171 males and 290 females) the bilateral DRN-hippocampal tract was successfully segmented. A significant regression equation was found ($F(2, 458)$, $p<1e-06$) with an R^2 of 0.055 (Figure 5). The predicted FA of the DRN-hippocampal tract is equal to 0.023 + 0.00046 (age) – 3.84 (sex).

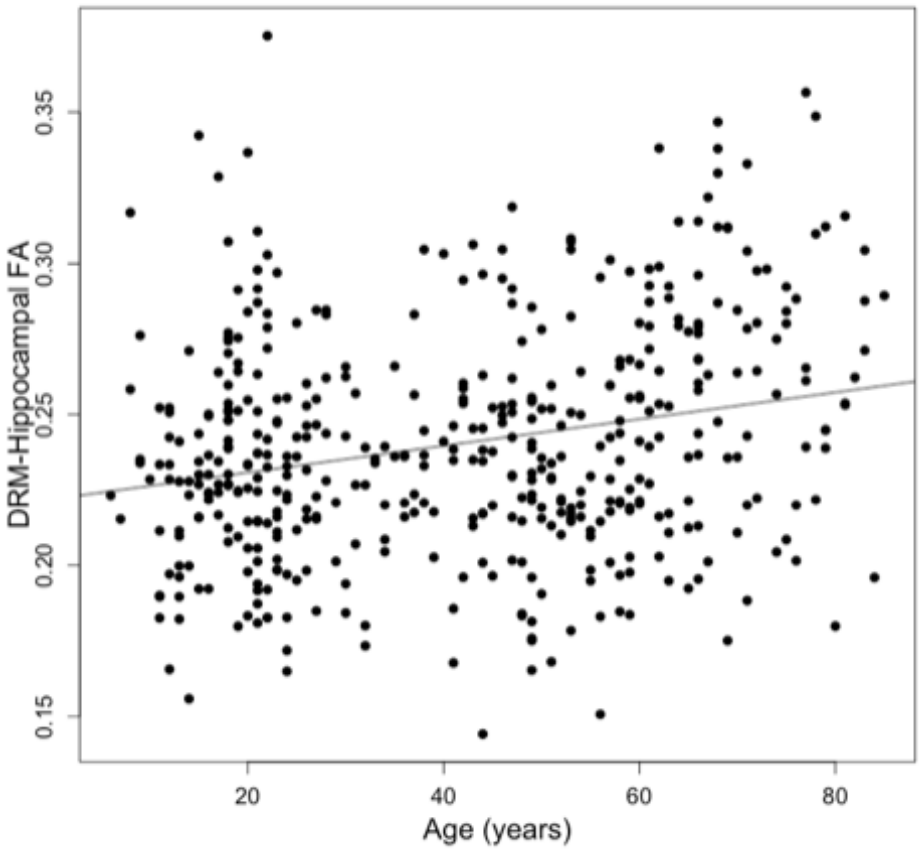

Figure 5: This figure represents the regression line for age versus DRN-hippocampal FA. The regression line equation is y=0.023 + 0.00046 (age) – 3.84 (sex).

DISCUSSION

The purpose of this study was to use diffusion tensor imaging to gain insight into the effects of aging on the raphe-hippocampal tract. The tract plays a key role in regulating mood through the production of the neurotransmitter serotonin. The results of the study show that with aging, the fractional anisotropy increases. This means that integrity of the dorsal raphe nucleus-hippocampal tract is increased throughout age. Because of this increase in integrity, there is a disruption in normal brain function. Fractional anisotropy or FA can also be considered as a measure of connectivity in the brain, meaning connectivity was increased in this region. This increase in connectivity is believed to also lead to decreased connectivity in regions outside of the inner forebrain.

When further investigating the connectivity between the dorsal raphe nucleus and the hippocampus, there appeared to be a correlation between increased connectivity and baseline depression. This means that there is a positive correlation in depressed young adults (24 plus or minus 4 years) and increased connectivity in the dorsal raphe nucleus with the hippocampus. Using this information from the study, it can be hypothesized that an increase in FA in the DRN-hippocampal tract as one ages could mean that people are becoming more depressed from this increase (Anand et al). The increase may be causing a shift in brain activity from a balance of active neurons in all regions to more activity taking place in the center of the brain as compared to later regions.

This dysconnectivity in regions beyond the hippocampus can be seen between the DRN and posterior cingulate, showing signs of late life depression. This finding was shown in a study done on adults with early Alzheimer's, mild cognitive impairment, and

normal controls. The posterior cingulate is a portion of the limbic system responsible for processing emotions, regulating behavior, and memory retrieval. This follows along with the assumption that more connectivity within the hippocampus is resulting in less activity outside of the structure. When there are alterations within the brain like this, it can result in imbalances. Since the hippocampus plays such a large role in mood stability, depression has been seen to be one of the leading causes of this change in brain activity. This dysconnectivity has also been seen in humans exhibiting early Alzheimer's disease, resulting in cognitive impairments (Zhou, et al).

Connectivity in the hippocampus cortex is seen to increase with age, which can then increase the function of memory during true and false retrieval of information. This type of memory retrieval does not pertain to the aforementioned decrease in memory from reduced connectivity as it is only referring to true or false questions. However, this finding that age alludes to stronger coupling with the parietal and dorsolateral prefrontal cortex can provide insight into the hippocampus also increasing its connectivity with the dorsal raphe nucleus. Although the hippocampus coupling to the aforementioned lobes is in regard to memory, it is still important to note the strengthening in connectivity as one ages (Paz-Alonso et al).

To further explain how an increased connectivity could be causing age-related problems, one must understand the role of the hippocampus. The hippocampus plays a role in emotion and memory, connecting to a wide variety of structures. It connects to the default mode network which is a network of brain regions that are active when one is not focused on the outside world (Default Mode Network). Thus, the hippocampus connects to these structures that pertain to memory. With age, it has been seen that the connectivity

is reduced to the DMN but increased between the lateral hippocampus. This then has resulted in reduction of memory which can be seen in a study done on adults between the ages of 25 and 80 years old. Overall, an increase in hippocampus connectivity means a decrease in interactions with other brain structures (Salami et al).

This then allows for the speculation that because the dorsal raphe nuclei connection to the hippocampus is increasing in fractional anisotropy, there could be decreases in surrounding regions. This leads one to believe that this could be the reason for aforementioned depression problems occurring when connectivity is increased in these forebrain regions. The dysconnectivity between the dorsal raphe nuclei and the posterior cingulate cortex accounts for a disruption in the serotoninergic input to the posterior cingulate cortex. This means less serotonin is making its way out of the DRN-hippocampal tract resulting in a decrease in mood stability and an increase in depression (Ikuta, et al).

These changes in connectivity show how impactful serotonin is as a neurotransmitter. With alterations occurring in the brain as one ages, it would make sense that mood and memory problems would begin happening. Because the DRN-hippocampal tract is located so deeply within the brain near the brain stem, it is one of the more important structures. The brain develops so that the most useful structures, those dealing with emotion, memory, wakefulness, etc., are buried. Yet, the regions just inferior to the skull are not involved in these higher cognitive processes. It may be hypothesized that as one ages the brain finds it more necessary to focus energy on these interior structures. Thus, the brain is increasing activity here while compromising activity for regions beyond the raphe nucleus and hippocampus.

CONCLUSION

Using diffusion tensor imaging allowed for analysis of the raphe-hippocampal tract in brains varying from ages 6 to 85 years old. The results show an increase in fractional anisotropy meaning more firing of axons and therefore, more connectivity. Because this is where serotonin is made, it could be hypothesized that there is a greater production of this neurotransmitter potentially increasing mood with age. However, when further researching other studies, it appears that as connectivity increases in these internal brain structures, it is decreasing in the more external structures. Thus, with age, connectivity and activation shift. The brain begins to prioritize connections that are deeply internal and creates dysconnectivity with surrounding and external lobes. This is decreasing the connectivity in the pathway that serotonin travels, resulting in less serotonin making its way out of the deep interior of the brain.

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