Pinealon Improves Cognition and Performance in Healthy Adults

Introduction:

Pinealon, also known as "EDR," is a tripeptide composed of Glu-Asp-Arg, with a molecular weight of 418.41. It was originally isolated from cortexin [1], indicating that it is naturally present in bovine or porcine cortex, specifically those less than 1 year old. It is perhaps the single most powerful peptide in existence for improving cognition and performance in healthy people, as well as exerting potent geroprotective and neuroregenerative effects, even completely restoring damage from Alzheimer's disease and Huntingtons disease in vitro. Its actions within the body are mediated through ligand binding to various genes, resulting in the enhancement or inhibition of their expression.

Mechanisms of action:

- Pinealon has a binding site within GDF11, and since it has been noted to show similar benefits to GDF11, it may increase its expression[53]. GDF11 is considered a rejuvenation factor in old age[54], retards the aging process in male mice[55], reduces depression phenotype in old mice by means of neural autophagy[56], and is necessary for stem cell production and DNA repair. This could potentially limit lifespan as GDF11 levels fall to

near 0 at a mean age of 73.71 years old[69]. GDF11 is shown to increase hippocampus and cortex vasculature in old mice when administered systemically but does not cross the blood-brain barrier (BBB) in appreciable quantities[71]. It shows benefits in spinal cord injury[73]. Higher GDF11 is associated with reduced incidence of cardiovascular events and death[72], and it regenerates myocardial tissue after ischemic injury[75]. GDF11 is also a positive regulator of muscle growth[60] and reverses age-related dysfunction in muscle[74]. It increases wound healing in diabetic mice[76], shows anti-aging benefits to the skin[77], and shows promise in AD[78].

- Pinealon binds to the histone H1.3 protein and increases the expression of the FKBP1b gene[46]. FKBP1b has been shown to reverse age-related intracellular Ca2+ dysregulation, cognitive and memory impairments in aging rats, and restore the expression of 872 out of 876 genes in the direction opposite of aging in the hippocampus of mice, which are associated with structure categories including cytoskeleton, membrane channels, and extracellular region[49]. Early AD also shows downregulated FKBP1B[47,48].

- Pinealon significantly upregulates PPARA/G genes in humans and improves the performance of wrestlers[11,50]. The PPARA/G genes have strong associations with athletic performance[58]. PPARG upregulation is a potential target to benefit spinal cord injury[82]. PPAR gene polymorphisms associated with the "g" allele result in decreased function and have a high correlation to reduced hand grip strength[89], increased risk of breast cancer[90], and AD[51]. PPARA also has an important role in

regulating autophagy and clearing AB plaques by increasing the microglia and astrocytes around the AB plaques and enhancing the formation of autophagosomes[61].

- Pinealon upregulates HSPA1A by about 3x in humans, though the dosage used was not specified [11,50]. It codes for heat shock protein 70 and is associated with the learning process in mice put through the Morris water maze [88]. It improves insulin sensitivity and decreases lipids, including within the brain [52]. It improves wound healing in vivo [85]. It prevents aggregation of misfolded proteins such as what occurs in AD, Huntington's disease, and PD [83], possibly partially through decreased cdk5 [96] which also improves resistance to oxidative stress and an increase in SIRT1 [98]. Lower levels of heat shock protein 70 are also associated with ADHD in children, hinting that its upregulation may play a role in benefiting ADHD [97].

- Pinealon increases FNDC5 gene expression, by binding to the gene in 5 regions, which codes for Irisin[26]. Irisin is a positive regulator of muscle growth[59], restores nucleus pulposus cells in mice and stops disc degeneration[79], and protects against motor dysfunction in rats with spinal cord injury[80]. It has been shown to increase telomerase and therefore the Hayflick limit[26], as well as SIRT1[86], a longevity and anti-cancer pathway that activates caspases in cancer cells specifically but not healthy cells[87]. Irisin stimulates mitochondrial biogenesis and mitophagy[95], prevents mitochondrial damage in PD[84], and provides potent antioxidant effects and reduces ferroptosis in hypoxia[28].

- Pinealon is able to bind directly to CASP3[30] and reduce caspase 3 expression[31], which is a regulator of apoptosis under hypoxia[29], and it was found to be the best peptide tested for hypoxia[32]. Inhibition of caspase 3 results in the upregulation of mitochondrial complex 1 of the electron transport chain, leading to increased ATP[33]. In spinal cord injury, caspase 3 is upregulated and leads to cell death, suggesting that lowering it can assist in spinal cord injury[81]. In AD, caspase 3 activation has been shown to occur in dendritic spines in the hippocampus, leading to activation of calcineurin and phosphorylation of glur1, causing spine degeneration and memory deficits. Caspase 3 inhibition rescues these deficits[34]. Caspase 3 inhibition is also associated with pinealon's purported ability to regenerate the skin[70].

- Pinealon forms a hydrogen bond in the CCTGCC promoter region of the TPH1 gene, which increases its expression. This leads to increased serotonin synthesis within cultured cerebrocortical neurons of mice origin[2]. In humans, the TPH1 gene has been found to have low expression in the cortex but high expression in the midbrain, such as the raphe nuclei[3]. Therefore, it is logical to assume that increased serotonin synthesis would primarily occur in the midbrain in humans. For an overview of serotonin synthesis[4].

Pinealon enhances cognition, mood, TBI recovery, protects from hypoxia, reverses neurodegeneration, and improves physical

performance.

- Improved intellectual markers in male wrestlers and older men[11].
- Improved spatial orientation and attention in aging rhesus monkeys while increasing the speed of learning by 1.5x[10].
- Improved mice performance in the Morris water maze and was associated with increased Nr2a/Nr2b ratio. The enhanced memory was retained even after streptozotocin-induced diabetes[8].
- Improved rats' performance in the water maze in comparison to cortexin[9].
- Improved asthenic symptoms and psychoemotional state in workers under stressful conditions[12].
- In TBI, Pinealon improved memory, lowered intensity and duration of headaches, increased emotional stability, and improved sense of well-being and being rested after a night's sleep, accounting for Pinealon's regenerative properties[57].
- Protects rat offspring from prenatal hyperhomocysteinemia[66], prenatal hypoxia[67], and in vivo hypobaric hypoxia, doubling the time needed for rats to reach respiratory arrest[68].
- Pinealon is shown to completely reverse the damage to mushroom spines in hippocampal cultures treated with Aβ42 in the amyloid synaptotoxicity AD model[23], completely restores the damage to dendritic spines in MSN's in an in vitro model of Huntington's disease[24], and improves locomotion and accuracy

of movements in a flying insect model of Parkinson's disease[25].

- Increases athletes' performance, breath-holding time, and decreases markers of biological aging[11].

Pinealon vs SSRIs:

Pinealon only enhances the synthesis of serotonin through TPH1, rather than inhibiting SERT. It may have a low incidence of sexual side effects because TPH1 is expressed in the hypothalamus[3], where it's shown to be a potential target to decrease neuroendocrine disruption[5]. SSRIs decrease rats' performance in the Morris water maze test[6], which is used to evaluate spatial memory and learning. SSRIs are generally associated with memory impairments in humans, though not always[7]. Pinealon has been shown to improve healthy mice and rats' performance in the water maze test[8,9] and improve some intellectual functions in wrestlers[11].

ERK1/2 activation:

Serotonin signals through GPCRs to increase cAMP levels[13], which activates PKA, which phosphorylates CREB. CREB plays a role in LTP, which is believed to be a cellular basis for learning and memory[14]. PKA can activate the ERK1/2 pathway directly[15], or it can be activated in some 5-HT receptors through GPCRs, β-arrestins, and small GTPases[15]. ERK1/2 is not associated with an increase in inflammatory

cytokines, unlike other MAPKs such as p38 and JNK[16]. Irisin and ERK1/2, which pinealon also activates directly, can each reinforce the other[27,41].

Activation of ERK1/2 influences cellular redox balance by activating NRF2, which in turn activates heme oxygenase 1, glutathione, catalase, and superoxide dismutase, all of which are antioxidants[17]. Pinealon has been shown in a study of cerebellar neurons to activate ERK1/2, possibly through the mechanisms explained above, though it could be multifaceted, and this activation is at least partially responsible for protecting those cells in a dose-dependent manner from hydrogen peroxide[18]. However, pinealon's speculated ability to bind to three regions in the GPX1 gene, which code for glutathione, and regions in the SOD1 gene which code for superoxide dismutase, may also contribute to its demonstrated antioxidant effect[19, table 1].

Pinealon in AD:

The above study showing protection from H2O2 is relevant to AD, as oxidative stress is one of the theories implicated in AD[20], and H2O2 is generated during the early stages of aggregation of amyloid plaques associated with AD[21]. According to the A1 astrocyte theory, damage caused by amyloid plaques can trigger microglia to release pro-inflammatory cytokines, chemokines, and ROS, thereby leading to A1 type astrocytes, which ultimately cause neurons and oligodendrocytes to self-destruct. This phenomenon is also seen in Parkinson's disease and Huntington's disease[22].

The hippocampus is the main brain region where neurogenesis can still occur throughout adulthood. It has been found that reductions in hippocampal neurogenesis may contribute to cognitive impairments, tau hyperphosphorylation in neurons, and compromised hippocampal circuitry in Alzheimer's disease[39,40]. When Aβ42 peptide is added to a culture of neurons, inhibition of ERK1/2, PI3K/Akt, and consequently BDNF occurs, which leads to mitochondrial dysfunction, secretion of pro-inflammatory cytokines, and cell death[45]. GDF11 improves neurogenesis and vascularization[71,78], heatshock protein 70 prevents aggregation of misfolded proteins[83], PPARA improves the correct functioning of microglia and astrocytes[61], non-amyloidogenic ERK1/2[35,36] stimulates neurogenesis in the hippocampus, and Irisin additionally activates STAT-3 and BDNF in the hippocampus[41,42]. STAT-3 activation has also been shown to reverse cognitive deficits in AD by increasing NMDAR expression and synaptogenesis, which is inhibited in AD by increased oligomeric amyloid beta peptide that causes internalization of the receptor and weakens synapses[43,44]. The ERK1/2 pathway also stimulates oligodendrocytes to myelinate axons, protecting neurons[37]. Myelination is reduced in Alzheimer's disease and ERK1/2 restores myelination[38]. It is through these mechanisms and all of the above targets, such as GDF11, FKBP1B, HSPA1A, and PPARA/G, that may contribute to the complete regeneration seen in Ab42 toxicity model.

Pinealon in Parkinson's:

As for how Pinealon improved symptoms in Parkinson's disease[25] and

increased DOPA and Dopamine[65], there are two potential mechanisms outside of the earlier noted regenerative abilities. Pinealon was shown to have a binding site within the CALM1 gene[64], which codes for calmodulin. The binding analysis did not state whether it increased or decreased its activity. Since calmodulin can bind to Gi proteins in the D2 receptor and reduce its signaling through a cAMP mechanism, it is possible that Pinealon decreased CALM1 and hence led to an increase in D2 signaling[62]. This would explain the enhanced locomotion and accuracy of movements witnessed in flying insects. However, there is also the fact that PPARA regulates cholinergic-driven activity of midbrain dopamine through a mechanism involving α7 nicotinic receptors[63]. This would also make sense as α7 agonists showed benefits in PD.

ROA and Dosing:

Pinealon has been studied orally and intramuscularly; however, I believe that intranasal administration may be necessary to get the most out of the cognitive benefits. Considering GDF11 cannot cross the BBB in appreciable quantities, it makes sense that directly increasing its expression in the brain, perhaps through Pinealon, could lead to farther-reaching benefits than what were seen in neuro-studies. Furthermore, oral Pinealon may result in increasing the expression of the TPH1 gene in the gut, which can potentially decrease bone mass[91] and hinder metabolic function[92], even though the increase in irisin would increase bone mass[93] and improve metabolic function[94]. Therefore, in my opinion, it would be best to avoid oral Pinealon.

Hence, I highly recommend dosing through either subcutaneous/intramuscular injection or a nasal spray. The dosages used in the intramuscular studies for TBI were up to 5mg daily, and this dose was shown to provide more benefits than lower doses. As for nasal spray, I can only share anecdotes from myself and others I've had try it, and we have found the best doses to be 1-3mg daily.

-Brenden Henry

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forms stable complexes. In the *TPH1* and *CALM1* genes , 1 binding site was found, and in the *VIM1* gene , 2 binding sites. Analysis of the promoter regions of genes encoding proteins that regulate the functional and antioxidant activity of cells (PPARA, PPARG, HSPA1A, SOD2, GPX1) also indicates the presence of binding sites for Pinealon. When studying the binding of the peptide to FITC-labeled histones, its interaction with the histones H1.1, H1.3, H1.6, H2b, H3, and H4 was found. These data were confirmed by the results of molecular modeling. Studies of Pinealon showed its antihypoxic, antistress, and neuroprotective effects [31].

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sensitive chromato-mass spectrometry revealed the Glu-Asp-Arg (EDR) tripeptide, which was named

Pinealon, in the drug Cortexin [50]. The tripeptide has a neuroprotective effect in rat cerebral cortex cell culture, reduces the level of neuronal apoptosis and increases serotonin synthesis. The addition of the peptide to a culture of striatal neurons obtained from YAC128 mice (a model of Huntington's disease) and to a culture of hippocampal neurons obtained from mice of the C57BL/6 line and PS1-M146V KI line (a model of Alzheimer's disease) resulted in statistically a significant increase in the number of spines of dendrites of neurons in comparison with the control. This indicates the ability of Pinealon to restore the neural network of the brain in neurodegenerative pathology [51]. Administration of the peptide to animals contributed to an increase in the content of adrenergic neurotransmitters: DOPA and dopamine in the cerebral cortex, and adrenaline in the brain stem structures. The antioxidant effect of peptida was also revealed in the model of hyperhomocysteinemia. Pinealon also protects the fetal brain of a pregnant female rat during hypoxia. It was found that the peptide normalizes the formation of the ability to preserve memory in bees *(Aris mellifera* L.), increases locomotor activity in fruit flies *(Drosophila melanogaster)* in the Parkinson's disease model, and restores short term memory in the Agnst3 mutant, which, apparently, , is associated with a decrease in the expression of the *limk1 gene.* After the use of Pinealon in monkeys *(Macaca Mulatta),* the duration of learning was reduced (by 1.5 times in comparison with control indicators) and the stability of attention increased when searching for an informative feature in visual stimuli [52]. Molecular modeling revealed two binding sites for this tripeptide, d(CCTGCC)2 and d(CCAGC)2 , with these sites the peptide

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