INTRACEREBROVENTRICULAR AND INTRAHIPPOCAMPAL ADMINISTRATION OF THE SYNTHETIC $A\beta_{1-42}$ TO THE RAT BRAIN. CONNECTION OF DENDRITIC SPINE DENSITY AND SPATIAL MEMORY.

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INTRODUCTION: Alzheimer's disease (AD) is associated with an early memory loss and is the major cause of dementia in the elderly. AD is characterized by presence of senile plaques which play important role of the pathogenesis. The oligomerization and the aggregation of amyloid-beta (Aβ) is believed to be central event in the dementia. Aggregates of Aβ oligomers are neuro- and synaptotoxic. Dendritic spines are one of the prominent features of neurons and have been studied for over a century. The number and health of dendritic spines are correlated to the health and functionality of synapses; in hippocampal pyramidal cells there is a one-to-one correlation between spine number and synapse number.

AIM OF THE STUDY:E vaulate the behavioral effects of intracerebroventricular (ICV) and intrahippocampal (IHC) administered synthetic $A\beta_{1-42}$ and measure the spine density.

MATERIALS AND METHODS:

	INTRACEREBROVENTRICULAR INJECTION	**INTRAHIPPOCAMPAL INJECTION (He et al., 2011)**
SUBJECTS (WISTAR, CR)	n=25	n=23
MATERIALS	$A\beta_{1-42}$, 200 μM, 5 days aggregation (37 ºC), hydrocarbonated buffer (HCBS)	$A\beta_{1-42}$, 222 μM, 7 days aggregation (37 ºC), physiological saline
METHODS	Injected bilaterally, 7,5 μl per site	Injected unilaterally (right side), 10 μl
GROUPS	HCBS (n=12), $A\beta_{1-42}$ (n=13)	Physiological saline (n=12), $A\beta_{1-42}$ (n=11)
MORRIS WATER MAZE (MWM)	6-days MWM carried out on the 14th day after $A\beta_{1-42}$ injection 4 trials/day, 1 trial 90 sec, on the platform 10 sec	6-days MWM carried out on the 14th day after $A\beta_{1-42}$ injection 4 trials/day, 1 trial 120 sec, on the platform 10 sec

GOLGI IMPREGNATION: Colgi-Cox impregnation has been one of the most effective technique for studying the morphology of neurons. The technique has proven to be extremely reliable and sensitive for demonstrating dendritic spines.

SUBJECTS: Wistar (CR) rat (n=12, 3 per each group)
METHODS: FD Rapid GolgiStainTM Kit (FD NeuroTechnologies, Consulting & Services, Inc., USA) was used.

1. 100 μm coronal sections were cut with microtome (Zeiss Microm HM 650V)
2. Pyramidal neurons from the dorsoventral hippocampal CA1 were studied (mostly in stratum radiatum)
3. The spine density of the proximal apical dendrite area was analyzed (100-200 μm from soma)
4. 25 cells per animals were countered
5. ImageJ 1.44 software (National Institute of Health, Bethesda, USA) was used to determine the density of spines

BEHAVIORAL RESULTS:

Fig.1: ICV injection of $A\beta_{1-42}$ resulted learning deficit. Performance is expressed as the mean latency (±S.E.M.) to find the platform during the 6 days period. REPEATED MEASURES ANOVA: p=0.014, F=5.252.

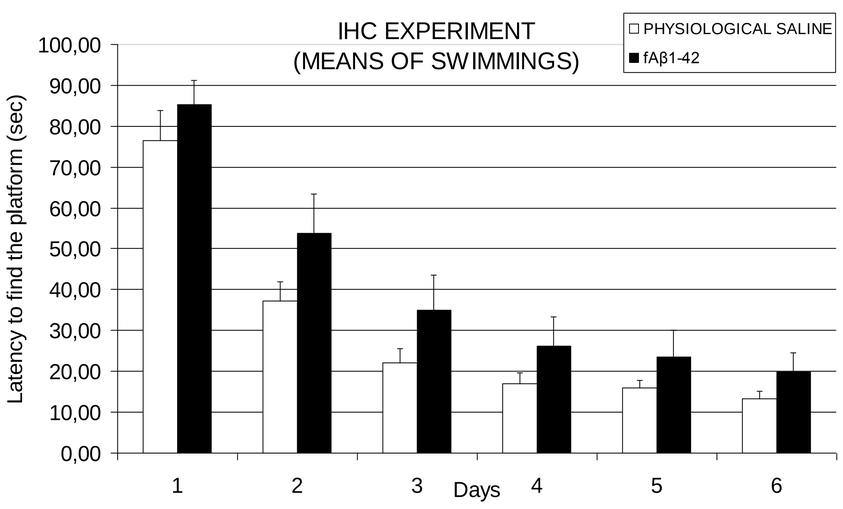


Fig.2: IHC injection of $fA\beta_{1-42}$ resulted learning deficit. Performance is expressed as the mean latency (±S.E.M.) to find the platform during the training. REPEATED MEASURES ANOVA: p=0.013, F=6.450.

GOLGI STANING RESULTS:

Fig.3: Apical dendritic spine density analysis revealed that the amyloid treatment induced a reduction in spine density (INDEPENDENT SAMPLE t-test: t=-13.708, df=22, *P<0.0001). Performance is expressed as the mean (±S.E.M.).

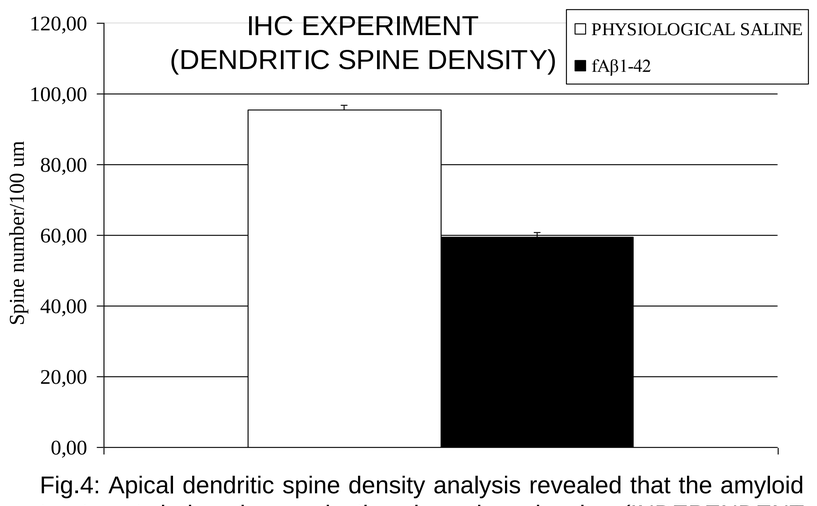
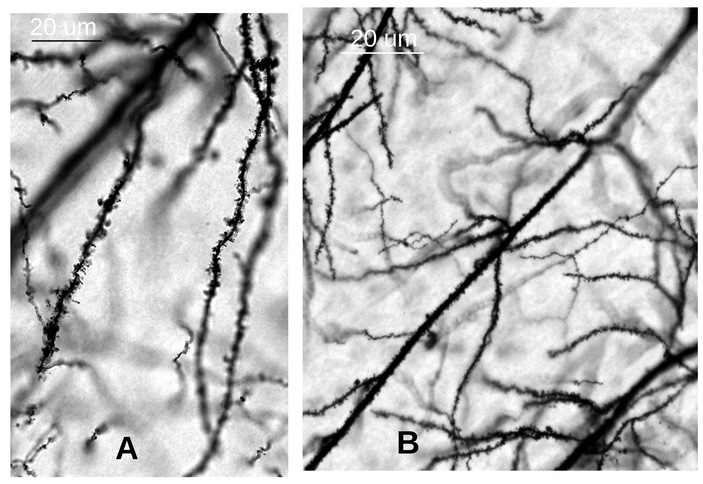
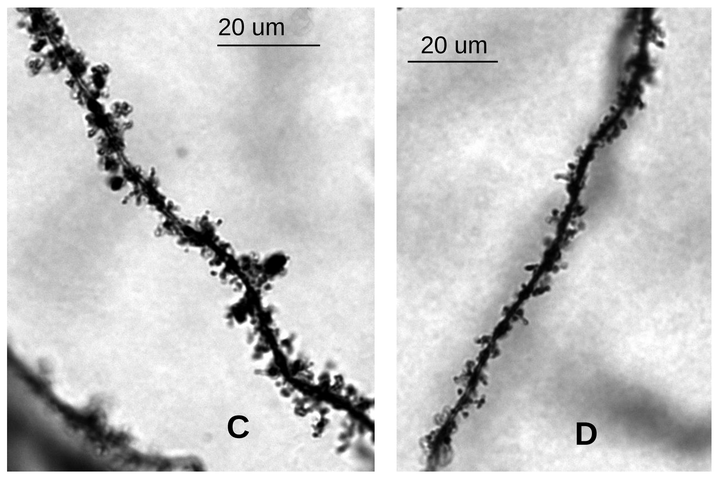


Fig.4: Apical dendritic spine density analysis revealed that the amyloid treatment induced a reduction in spine density (INDEPENDENT SAMPLE t-test: t=-11.303, df=22, *P<0.0001). Performance is expressed as the mean (±S.E.M.).

ICV GOLGI STANING:

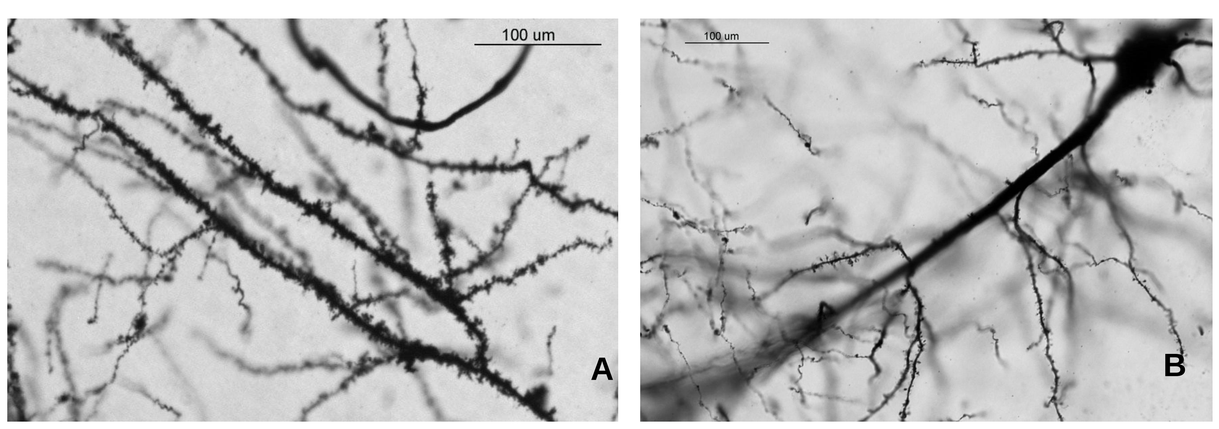


Picture 1: Golgi staining revealed changes in spine density. Photomicrograph of the CA1 subfield pyramidal neuron from a control (A) and an amyloid-treated (B) animal. Magnification 200x.

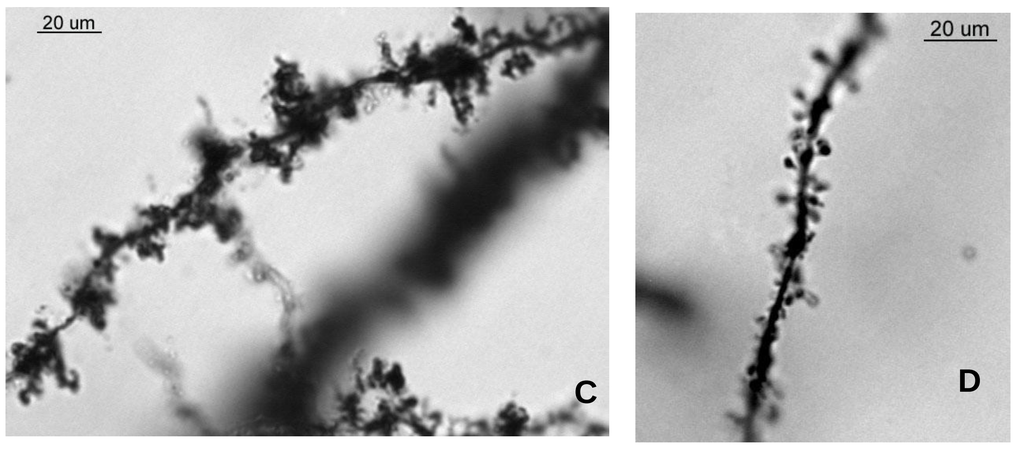


Picture 2**:** Photomicrograph of oblique dendritic segments from hippocampal CA1 pyramidal neurons of control rat (C) and amyloid-treated (D). Magnification 1000x.

IHC GOLGI STANING:



Picture 3: Golgi staining revealing changes in spine density. Photomicrograph of the CA1 subfield pyramidal neuron from a control animal (A) and an amyloid-treated (B). Magnification 200x.



Picture 4**:** Photomicrograph of oblique dendritic segments from hippocampal CA1 pyramidal neurons of control rat (C) and amyloid-treated (D). Magnification 1000x (D).

CONCLUSIONS:
IN ICV AND IHC EXPERIMENTS SPATIAL MEMORY DEFICIT WAS DETECTED IN MORRIS WATER MAZE TEST. IN THE $A\beta_{1-42}$-TREATED GROUPS THE LATENCY TO FIND THE PLATFORM WAS SIGNIFICANTLY HIGHER COMPARED TO THE CONTROL GROUPS.
IN BOTH EXPERIMENTS THE TREATMENT OF $A\beta_{1-42}$ RESULTED A SIGNIFICANT DECREASE OF SPINE NUMBER COMPARED TO THE CONTROLS. THE NUMBER OF DENDRITIC SPINES ARE CORRELATED TO THE HEALTH AND FUNCIONALITY OF SYNAPSES AND THE SPATIAL MEMORY FUNCTION.

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