FULL REPORT

ISN-CAEN Award

Visit by the applicant to another laboratory (CATEGORY 1A)

Project: Dopaminergic stimulation as a mechanism for preventing the impairment caused by Amyloid-β peptide in different models of Alzheimer's disease

Host Laboratory: Dr. Nibaldo Inestrosa, Full Professor
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Dopaminergic stimulation as a mechanism for preventing the impairment caused by Amyloid-β peptide in different models of Alzheimer's disease

● Background

Alzheimer's disease (AD) is the most common form of dementia in elderly people. It affects more than thirty-five million people worldwide, and this number is expected to increase in the next decades (Alzheimer's Association, 2013). The disease is clinically characterized by progressive cognitive decline and memory loss, which appears as a result of synaptic and neuronal damage (Viola et al., 2008). The identification of the amyloid β peptide as the principal component of senile plaques in AD brains in the late 1980s (Joachim et al., 1989) led to the amyloid cascade theory, by which the deposition of toxic amyloid fibrils are responsible for the AD pathology. Although plaques may have a role in AD pathology, some neuropathologists rejected the hypothesis, because there is a poor correlation between plaque load and cognitive impairment (Galasko et al., 1994). Some experiments in animals models also failed to correlate memory impairment with amyloid pathology (Haass and Selkoe, 2007).

During the past decade, much of the focus in AD pathogenesis has turned to soluble oligomers of the Aβ peptide (AβOs). It is known now that AβOs are neurotoxins that accumulate in AD brains (Xia et al., 2009) and have been considered as the actual pathogenic culprit of AD (Mucke and Selkoe, 2012). The so-called oligomer hypothesis of AD memory impairment is now supported by strong evidence (Ferreira and Klein, 2011).

Dopamine is a neurotransmitter produced in the midbrain that plays a number of important roles in the brain and body. In the brain, dopamine is produced in the *Substantia Nigra* and in the Ventral Tegmental Area, and its best known for its connection with the sensory-motor cortex, mediating reward and motivation circuits (Beaulieu and Gainetdinov, 2011). Very recent studies have established a link between dopaminergic transmission and memory, mainly in the hippocampus and in the pre-frontal cortex (PFC) where it is involved especially in working memory and long-term memory (LTM) mechanisms (Puig et al., 2014; Rossato et al., 2009). It is well established that interaction between D_1 and NMDA receptor signalling is essential for different types of memory formation, mainly in the PFC (Castner and Williams, 2007). It is also known that glutamatergic afferents and dopaminergic terminals co-localize in the PFC, suggesting the existence of a presynaptic site of modulation that can influence the release of both neurotransmitters (Zheng et al., 1999).

Recent studies have established a relationship between morphological and functional changes occurring in the dopaminergic system of AD patients' brains (Trillo et al., 2013). Notably, dopaminergic transmission has been hypothesized as a new player in the pathophysiology of AD (Martorana et al., 2009), and AD patients are already being treated with dopaminergic agonists with positive results in restoring LTP-like cortical plasticity (Koch et al., 2014). In animal models of AD, it has been shown that dopaminergic agonists can reduce amyloid deposition and may also improve memory, but the mechanisms underlying this effects are still not known (Koch et al., 2014).

In 2011, our group showed that activation of D_1/D_5 receptors in hippocampal cultures prevents a reduction in surface levels of AMPA and NMDA receptors induced by AβOs, reinforcing the relevant link between dopaminergic and glutamatergic neurotransmission in AD models (Jürgensen et al., 2011).

● **Objectives**

In this context, we hypothesized that dopaminergic transmission, via D_1 receptor activation, may counteract the deleterious effects of AβOs on cognition and memory. Previous work from our group, using mice injected with a single dose of AβOs in the lateral ventricle of the brain, have demonstrated that AβOs acutely impair different types of memory, such as object recognition and fear conditioning (Figueiredo et al., 2013; Lourenco et al., 2013). Our data currently supports the notion that mice treated with D_1 agonists are protected from memory loss caused by AβOs. For this proposal, we planned to expand our approach with D_1 agonists to APP/PS1 mice, an animal model of AD which accumulates high levels of Aβ.

Our specific goals were: (1) to verify if treatments with D_1 agonists are able to restore the memory loss expected of transgenic AD mice; (2) to understand the possible mechanisms by which D_1 activation may lead to protection against the neurotoxic effects of AβOs; (3) to verify if an interaction between dopaminergic and glutamatergic neurotransmission has a role in such protection.

With these goals in mind, APP/PS1 or wild-type (WT) mice were treated for several days with D_1 agonists and then cognitive evaluation was carried out by means of the novel object recognition task and memory flexibility navigation task. After the memory tests, the hippocampi and pre-frontal cortices of the animals were collected for biochemical analysis of dopaminergic signalling. In another set of experiments, pre-frontal cortices and hippocampi of APP/PS1 and WT animals treated with D_1 agonists were dissected and used to evaluate changes in long-term potentiation (LTP) by electrophysiology.

● **Experiments performed**

<u>Behavioural analysis</u>

To evaluate if treatment with a D_1 agonist is able to reverse some of the cognitive deficits usually seen in APP/PS1 mice, we treated the animals with the D1 agonist SKF38393 for one week, with daily doses of 10 mg/kg and then performed the novel object recognition test. The test was performed in an open field arena with the floor divided by lines into nine equal rectangles. Test objects were made of glass or plastic and had different shapes, colors, sizes, and textures. During behavioral sessions, objects were fixed to the box using tape to prevent displacement caused by exploratory activity of the animals. None of the objects used in our experiments evoked innate preference. Before training, each animal was submitted to a 5 min-long habituation session, in which they were allowed to freely explore the empty arena. During habituation sessions, the total distance traveled and time spent in the center of the arena were measured for each animal. Time spent in the center is also a good parameter for anxiety behavior. Animals that spend more time in the central part are usually less anxious comparing to those that spend more time in the corners. No significant differences were found between experimental groups.

The time spent exploring each object was recorded during habituation sessions. Two hours after, animals were again placed in the arena, where one of the two objects used in the training session was replaced by a new one. Again, time spent exploring the familiar and novel objects were measured. Results were expressed as percentage of time exploring each object during the training or test session and were analyzed using a one-sample Student's *t* test comparing the mean exploration time for each object with the fixed value of 50%. By definition, animals that recognize the familiar object as such (i.e., learn the task) explore the novel object more than 50% of the total time.

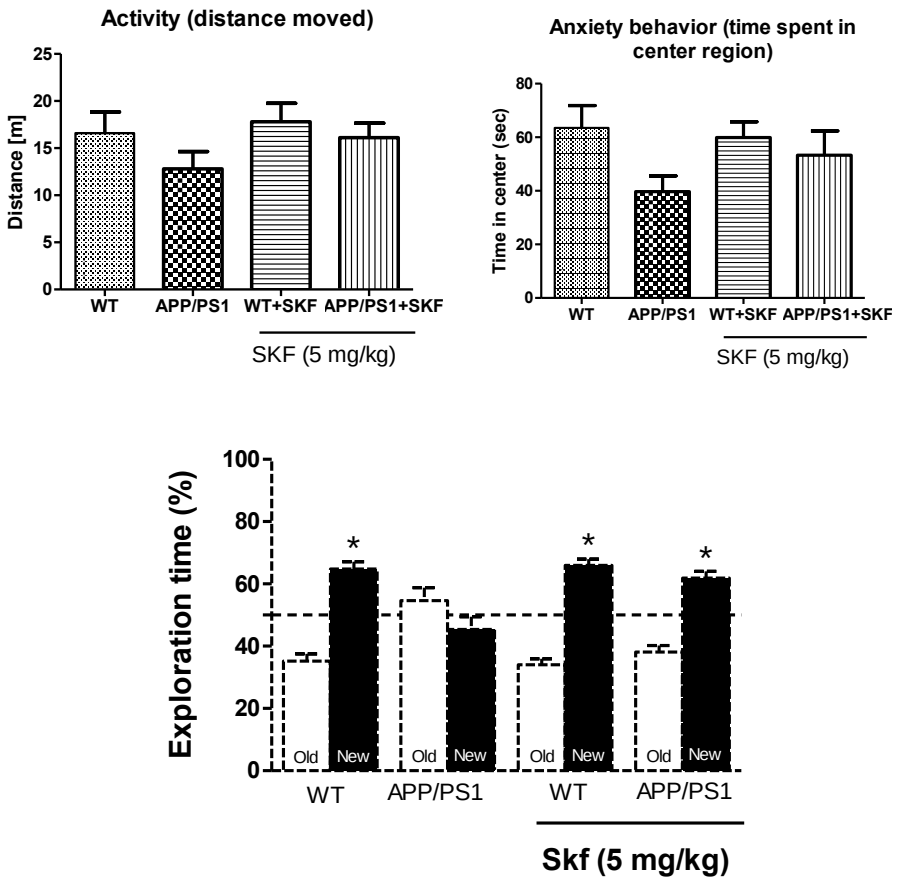

Fig 1. D1 agonist treatment is able to restore memory deficits in APP/PS1 mice.
Eight-month-old APP/PS1 mice were purchased from the Jackson Laboratory. Wild-type (C57/Bl6) mice were obtained from the Animal Housing Facility of the Facultad de Ciencias Biologicas (P.Universidad Catolica de Chile). N=9-10 per group

A second behavioral test performed with the APP/PS1 and WT animals was the memory flexibility test, described in recent papers (Inestrosa and Toledo, 2008; Vargas et al., 2014). Animals were trained in a circular water maze (1.2 m diameter, opaque water, 50 cm deep, 19–21°C, 9 cm platform 1 cm below water colored opaque with nontoxic white paint) for 4 days, with a new platform location each day. Each animal was trained for one pseudo-random location of the platform per day, for 4 days. Training was conducted up to 15 trials per day, until the criterion of three successive trials with escape latency below 20s was met. On completion of testing, the mouse was removed from the maze, dried and returned to its cage. The following day, animals were tested for the next location. Data were collected using a video tracking system.

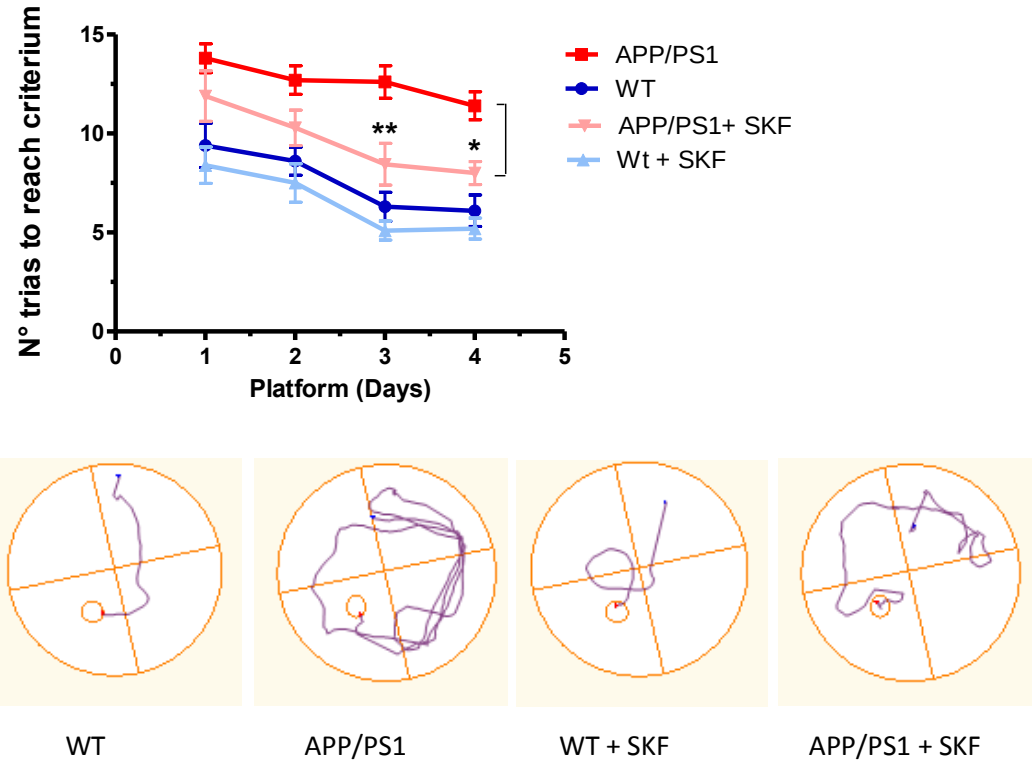

Fig. 2. D1 agonist is able to prevent the loss of spatial memory in APP/PS1
An increase in the number of trials necessary to reach the criterion is observed in APP/PS1 animals versus wild-type animals. Treatment with SKF38393 (daily doses of 10 mg/kg) reduces the number of trials necessary to reach the criterion (A). Representative swimming path of one animal from each group, in the last day of testing (B). N=9-10 per group

Electrophysiology

For electrophysiology experiments, 400µm-thick hippocampal slices from WT or APP/PS1 mice (treated or not with SKF38393) were allowed to recover for 1 hour after sectioning and then stimulated with tetanic stimuli and recorded for at least 100 min.

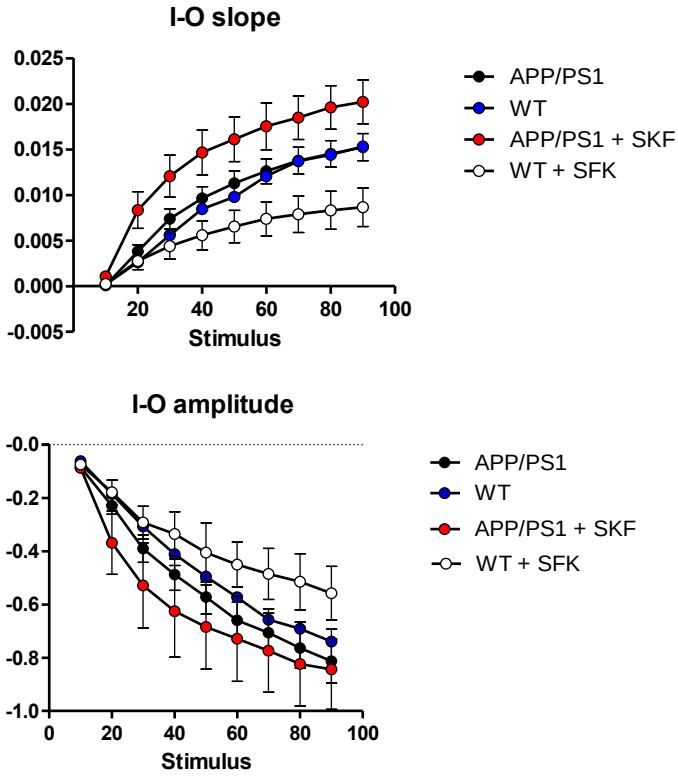

Fig. 3. Input-output field excitatory post synaptic potential (fEPSP), in response to increasing stimuli
The first graph shows the quantification of fEPSP slope, and the second graph the quantification of fEPSP amplitude. Interestingly, graphs show an increase in synaptic strength in the APP/PS1 group treated with SFK38393. The bottom graph shows a similar trend. N=4-5 per group

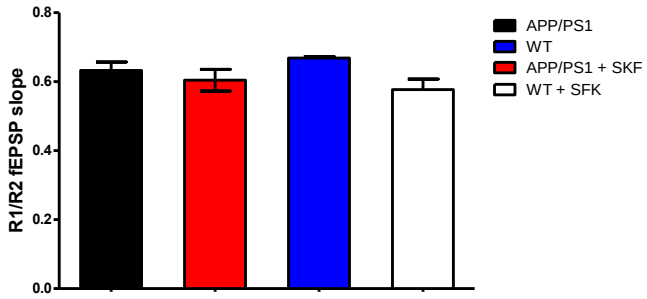

Fig. 4. Paired pulse facilitation (PPF)

The graph shows that PPF is unchanged in all experimental conditions. One interpretation is that, in this timeframe, the presynaptic compartment is likely unaffected by the APP/PS1 genetic background, or the drug treatment. N=4-5 per group

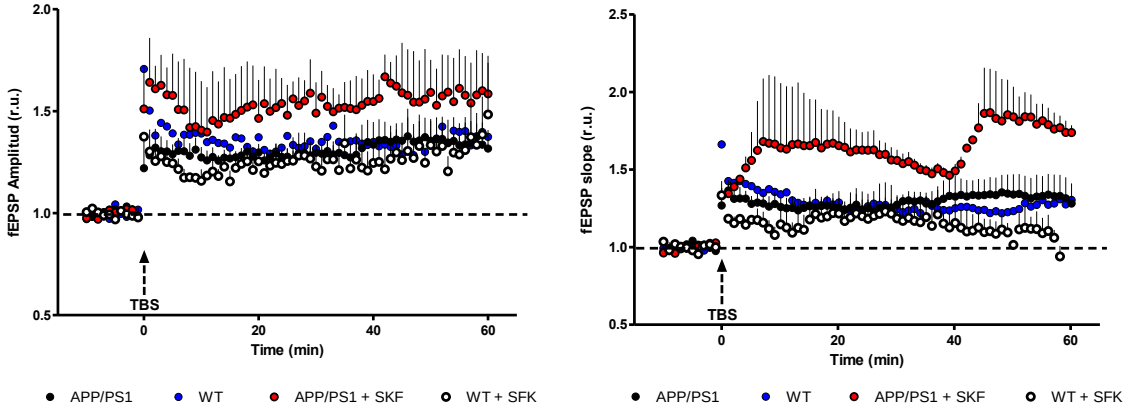

Fig. 5. LTP experiments

LTP induced by theta burst stimulation after 10 mins of stable recording (baseline). APP/PS1 mice treated with the D_1 agonist show an increase in the magnitude of LTP compared with other groups. Additional experiments are currently being carried out in Dr. Inestrosa's lab, using WT and WT+SKF groups to complete this dataset. N=1-5 per group.

Biochemical analysis

Total levels of Aβ 1-42 were measured in all groups.

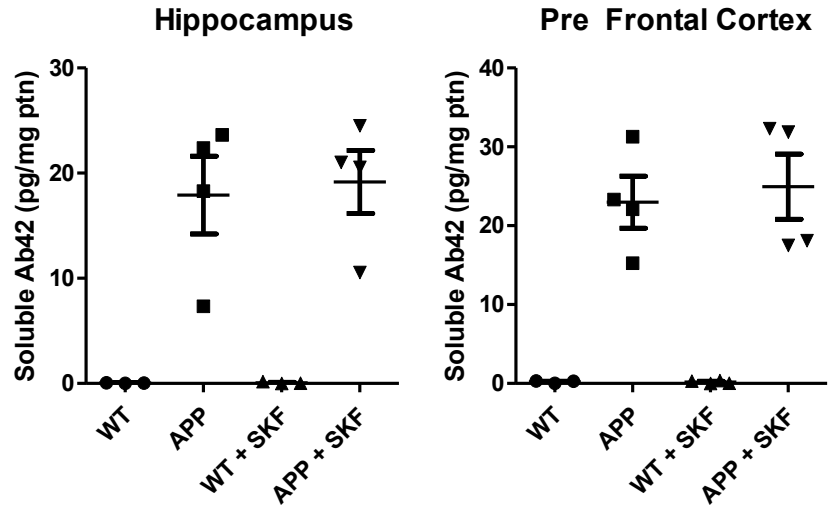

Fig. 6. Aβ 1-42 levels in treated animals
APP/PS1 mice have increased levels of $A\beta_{1-42}$, as expected. Treatment with the D1 agonist, SKF38393, was not able to reduce these levels, despite the significant behavioural effects. N=9-10 per group.

Additionally, BDNF levels were measured in all groups. BDNF is a neurotrophin, relevant to memory acquisition and consolidation. It is well known that enhancing dopaminergic transmission may increase BDNF levels through D_1-meadiated cAMP production, followed by PKA activation.

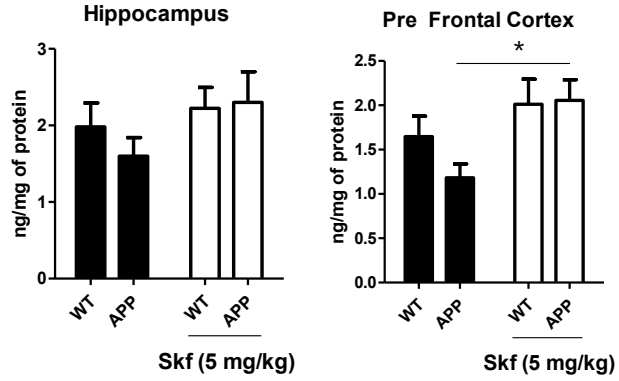

Fig. 7. BDNF levels in treated animals
No significant differences were observed in the hippocampus of WT x APP/PS1 mice, with or without SKF38393 treatment. In the prefrontal cortex, there was an increase in BDNF levels in both groups after treatments with the D1 agonist. N=6-7 per group.

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● **Outcome and benefit of the award**

I would like to thanks ISN-CAEN committe for the Travel Award 1A for a scientific visit to another lab. With the travel award, I was able to conduct a series of experiments relevant for my PhD thesis. I stayed at the laboratory of Dr. Nibaldo Inestrosa, participating in the actives of the Center for Aging and Regeneration (CARE) from the Pontifical Catholic University of Chile, in Santiago. It was an excellent opportunity to exchange ideas, learn new techniques and to work with a different animal model, that we do not have in Brazil. I have learned different behavioral experiments with the animals and the basis of electrophysiology records. The results obtained in these collaborations will be an important part of the manuscript that I am writing as a part of my PhD thesis. More than that, this was an excellent opportunity to exchange scientific ideas with Dr. Inestrosa and his team, and work in an excellent environment in the CARE and interact with people from there. I was trained in different protocols, which were very useful and definitely some of them I will be able to apply in my laboratory in Brazil. Hence I am truly grateful to ISN/CAEN for this fantastic support to my career development.

Best regards,

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