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Research Report

# Increased exploratory activity of APP23 mice in a novel environment is reversed by siRNA

Yann Senechal[b,1], Laetitia Prut[b,1], Peter H. Kelly[b], Matthias Staufenbiel[b], Francois Natt[b], Daniel Hoyer[b], Christoph Wiessner[b], Kumlesh K. Dev[a,b,*]

[a]Molecular Neuropharmacology, Department of Physiology, Trinity College Institute of Neuroscience, Trinity College Dublin, Dublin 2, Ireland
[b]Novartis Institutes for BioMedical Research, Basel, Switzerland

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ABSTRACT

Genetic abnormalities in *amyloid precursor protein* (APP) are associated with Down's syndrome and familial Alzheimer's disease where hallmark plaques contain Aβ peptides derived from APP. Both APP and its derivatives are implicated in neurodegenerative processes and may play important physiological and pathophysiological roles in synaptic function. Here, we show that young APP23 transgenic mice overexpressing human APP with the Swedish double mutation display altered novelty seeking behavior before the age of plaque onset. Using short interfering RNA (siRNA) targeted against APP, we investigate the direct contribution of APP and its derivatives to this behavioral deficit. After validating siRNAs targeting human APP *in vitro*, siRNAs were infused directly into the brain of APP23 mice for 2 weeks. Behavioral analysis shows that infusion of siRNA targeted against APP completely reverses increased exploratory activity in APP23 mice. Collectively, these data suggest that excessive APP and/or its derivatives, causes a hyperactive phenotype in APP23 mice when placed in a novel environment, which is fully reversible and not linked to plaque deposits.



## 1. Introduction

Alzheimer's Disease (AD) is characterized by progressive cognitive decline and histopathological hallmarks including neurofibrillary tangles and plaques composed of the proteolytic derivatives of Amyloid Precursor Protein (APP), Amyloid β (Aβ) peptides (Selkoe, 2001). It is important to note that APP may also play a physiological role in synaptic plasticity and hippocampal-dependent learning and memory (Senechal et al., 2006). Based on the link between mutations in the gene *app* and familial early onset AD (Tanzi and Bertram, 2005), a variety of transgenic mouse lines overexpressing different forms of human APP (hAPP) have been created as models of AD and studied behaviorally (Kobayashi and Chen, 2005). One of these transgenic mice, referred to as APP23, overexpresses human APP751 with the Swedish double mutation (K670N/M671L) under the control of a neuron-specific Thy-1 promoter, which induces a strong neuronal expression especially in the

* *Corresponding author*. Molecular Neuropharmacology, Department of Physiology, Trinity College Institute of Neuroscience, Trinity College Dublin, Dublin 2, Ireland. Fax: +353 21 427 3518.
E-mail address: k.dev@ucc.ie (K.K. Dev).

*Abbreviations:* APP, Amyloid precursor protein; AD, Alzheimer's disease; siRNA, short interfering RNA; CHO, Chinese hamster ovary; YFP, Yellow fluorescent protein

[1] Equally contributed to this work.

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hippocampus and cerebral cortex (Sturchler-Pierrat et al., 1997).

APP23 mice develop amyloid plaques in the neocortex and hippocampus starting at the age of 6 months, and plaque load increases with age (Sturchler-Pierrat et al., 1997). These plaques are accompanied by additional AD-like histopathological features including microglial reactivity (Stalder et al., 1999), dystrophic neurites and hyperphosphorylated tau (Sturchler-Pierrat et al., 1997; Sturchler-Pierrat and Staufenbiel, 2000). In addition to the parenchyma, amyloid deposition also occurs in vessel walls leading to cerebral amyloid angiopathy (Calhoun et al., 1999; Winkler et al., 2001). APP23 animals exhibit modest neuronal loss in the CA1 subregion of the hippocampus (Calhoun et al., 1998), a reduction of total cholinergic fiber length (Boncristiano et al., 2002) and decreased activities of acetylcholinesterase and choline acetyltransferase in basal forebrain nuclei (Van Dam et al., 2005). These animals also display progressive age-related behavioral and learning impairments in passive avoidance and Morris water maze (Van Dam et al., 2003; Kelly et al., 2003; Lalonde et al., 2002; Dumont et al., 2004). In addition, APP23 transgenic mice show overnight cage hyperactivity and altered exploration and activity levels in open field tests (Van Dam et al., 2003). Consistent with spatial memory deficits, APP23 mice are impaired in the acquisition phase of the Morris water maze task (Lalonde et al., 2002; Dumont et al., 2004). Thus, APP23 mice exhibit neuropathological and behavioral AD-like deficits which progress with age (Selkoe, 2001).

Since APP23 mice do not exhibit amyloid plaques before the age of 6 months (Sturchler-Pierrat and Staufenbiel, 2000), yet show increasing levels of APP and Aβ peptides at the age of 3 months (Capetillo-Zarate et al., 2006), we examined early behavioral deficits that could be caused directly by overexpression of APP and/or its derivatives. In the present study, to assess further the direct contribution of APP overexpression to the early behavioral deficits displayed by APP23 mice, APP was acutely downregulated in the brains of APP23 transgenic mice using a previously described siRNA-infusion approach (Senechal et al., 2007; Thakker et al., 2004). After siRNAs targeting human APP (hAPP-siRNAs) were validated *in vitro*, they were continuously infused for 2 weeks into three-month-old APP23 mice. Here we report the effects of APP knockdown by siRNA and confirm a direct role of APP/Aβ overexpression in

**Table 1 – Sequence of the siRNAs used in the human APP silencing study**

| siRNA name | Gene targeted | Sequence (5′–3′) | sRNA efficiency |
|---|---|---|---|
| Si1 | hAPP | AUGAGUUUCGCAAACAUCCdAT<br>TTUACUCAAAGCGUUUGUAGG | ++ |
| Si2 | hAPP | UUCACUAAUCAUGUUGGCCdAdA<br>TTAAGUGAUUAGUACAACCGG | ++ |
| Si3 | hAPP | UUGCUUGACGUUCUGCCUCTT<br>TTAACGAACUGCAAGACGGAG | + |
| Si4 | hAPP | UUCCUUGGUAUCAAUGCAGdGT<br>TTAAGGAACCAUAGUUACGUC | ++ |
| Si5 | hAPP | UUCUUGGCAAUACUGCAGGdAT<br>TTAAGAACCGUUAUGACGUCC | +++ |
| Si6 | hAPP | UCACAUCAAAGUACCAGCGdGdG<br>TTAGUGUAGUUUCAUGGUCGC | +++ |
| Si7 | hAPP | UUCGUUUCGGUCAAAGAUGdGdC<br>TTAAGCAAAGCCAGUUUCUAC | – |
| Si8 | hAPP | UUGCACCUUUGUUUGAACCdCdA<br>TTAACGUGGAAACAAACUUGG | + |
| Si9 | hAPP | UUCAUAUCCUGAGUCAUGUdCdG<br>TTAAGUAUAGGACUCAGUACA | ++ |
| Si10 | hAPP | UUUCCGUAACUGAUCCUUGdGT<br>TTAAAGGCAUUGACUAGGAAC | + |
| Si11 | hAPP | UUCCGUAACUGAUCCUUGGTT<br>TTAAGGCAUUGACUAGGAACC | ++ |
| Si12 | hAPP | AUCAGCUUUAGGCAAGUUCTT<br>TTUAGUCGAAAUCCGUUCAAG | + |
| Control siRNAs | | | |
| MM5 | hAPP mismatch | UUCUGGGCACUACGGCAGGdAT<br>TTAAGACCCGUGAUGCCGUCC | – |
| MM6 | hAPP mismatch | UCACCUCAACGUAACAGCGdGdG<br>TTAGUGGAGUUGCAUUGUCGC | – |
| siYFP | YFP | UUGAAGUUCACCUUGAUGCdCdG<br>TTAACUUCAAGUGGAACUACG | – |

Table indicating the siRNA sequences used in the study. Sequences of the antisense strand (upper sequence, 5′–3′ orientation) and of the complementary sense strand (lower sequence, 3′–5′ orientation) are indicated for each siRNA. Two-deoxyribonucleotide overhangs (dA, dG, dC or T) are placed at the 3′ end of each siRNA strand. The abbreviation hAPP stands for human APP. Underlined bases indicate mismatch harbored by the control siRNAs as compared to match siRNA. The efficacy of APP knockdown in our co-transfection assay is indicated as follows: – no effect, + to +++ indicates increasing APP silencing efficiency.