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Research Report

# Amyloid beta modulation of neuronal network activity *in vitro*

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ABSTRACT

*In vitro* assays offer a means of screening potential therapeutics and accelerating the drug development process. Here, we utilized neuronal cultures on planar microelectrode arrays (MEA) as a functional assay to assess the neurotoxicity of amyloid-β 1-42 ($A\beta_{42}$), a biomolecule implicated in the Alzheimer's disease (AD). In this approach, neurons harvested from embryonic mice were seeded on the substrate-integrated microelectrode arrays. The cultured neurons form a spontaneously active network, and the spiking activity as a functional endpoint could be detected via the MEA. $A\beta_{42}$ oligomer, but not monomer, significantly reduced network spike rate. In addition, we demonstrated that the ionotropic glutamate receptors, NMDA and AMPA/kainate, play a role in the effects of $A\beta_{42}$ on neuronal activity *in vitro*. To examine the utility of the MEA-based assay for AD drug discovery, we tested two model therapeutics for AD, methylene blue (MB) and memantine. Our results show an almost full recovery in the activity within 24 h after administration of $A\beta_{42}$ in the cultures pre-treated with either MB or memantine. Our findings suggest that cultured neuronal networks may be a useful platform in screening potential therapeutics for Aβ induced changes in neurological function.



## 1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder in which the loss of synapses and neuronal apoptosis in cortex and hippocampus lead to behavioral deficits such as memory deficits and cognitive impairment (Lublin and Gandy, 2010). Amyloid plaques which consist of aggregated fibrillar amyloid beta (Aβ) peptides are considered a pathological marker for AD

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(Benilova et al., 2012). Aβ peptides are produced by cleavage of amyloid precursor protein (APP) generating peptides of lengths ranging from 36 to 43 amino acids (Zhao et al., 2012). The major protein constituents of AD plaques are peptides of 40 and 42 amino acids in length or $A\beta_{40}$ and $A\beta_{42}$, respectively (Götz et al., 2011). The longer peptide, $A\beta_{42}$, has a high tendency to aggregate and it readily oligomerizes to form soluble dimers, trimers, and higher order oligomers (Citron, 2010; Mairet-Coello et al., 2013). The oligomers then aggregate further and form Aβ fibrils which constitute the amyloid plaques. However, prior work suggests that the most neurotoxic forms of Aβ are not the senile plaques or fibrillar Aβ but soluble Aβ oligomers (Bucciantini et al., 2002; Kirkitadze et al., 2002). In patients with AD, declines in cognition and memory appear correlated with increases in the fraction of soluble Aβ oligomer suggesting a dynamic equilibrium between multiple forms of the biomolecule (Rowan et al., 2003).

The exact mechanisms of neurotoxic effects of Aβ are not yet clearly understood. Formation of toxic pores in the cell membrane (Alarcón et al., 2006), increase in the intracellular oxidative stress (Nunomura et al., 2010), cell cycle re-entry (Seward et al., 2013), disrupting intracellular calcium release (Lazzari et al., 2014), and interruption of synaptic transmission (Eckert et al., 2008) are all among the proposed mechanisms. In addition, there have been numerous studies concerning the target receptor for Aβ oligomers. Aβ appears to interact with a wide range of cellular receptors including but not limited to nicotinic cholinergic receptors, glutamatergic receptors, ephrin-type B2 receptors, etc. (for review see Cheng et al., 2014; Mucke and Selkoe, 2012). With respect to synaptic transmission changes that are correlated with altered cognitive function, prior studies point to a role for N-methyl-D-aspartate (NMDA) receptors as either receptors for $A\beta_{42}$ or as an intermediary of its neuroactive effects (Deng et al., 2014; Ferreira et al., 2012; Texidó et al., 2011; Yamin, 2009).

To date, various transgenic animal models and mammalian cell assays have been utilized to identify potential therapeutics for AD (Gravitz, 2011; McColl et al., 2012; Wilcock, 2010). In particular, *in vitro* assays are advantageous for drug screening applications because they reduce animal usage, provide initial risk assessment, and thus have the potential to accelerate the drug discovery process (Harry et al., 1998). To this end, assays ranging from mammalian cells which accumulate Aβ (Haugabook et al., 2001) to human induced pluripotent stem (iPS) cells expressing APP (Yahata et al., 2011) have been proposed. However, most of the available *in vitro* preparations do not provide functional endpoints that capture physiologically relevant neuronal activity. In addition, the associated experiments are not designed to assess immediate effects of the oligomer (Tamburri et al., 2013). As such, a network-level functional assay which can provide reliable, fast, and high-content measures is desirable. An approach which has gained attention and interest as a platform for neuropharmacology and neurotoxicity testing involves the use of neuronal networks on microelectrode arrays (MEAs). Murine primary cultures derived from neural tissue form spontaneously active networks on substrate-integrated MEAs. Previous work has demonstrated successful applications of this platform as a biosensor in neuropharmacology (Johnstone et al., 2010; Keefer et al., 2001; Morefield et al., 2000; Xiang et al., 2007), assessing biocompatibility of novel materials (Charkhkar et al., 2014) and basic neuroscience (Bakkum et al., 2008; Hamilton et al., 2013; Wagenaar et al., 2005). This method is well established (Gross et al., 1985) and has been validated across different laboratories (Novellino et al., 2011). Compared to single-cell techniques such as patch clamp, the MEA method is non-invasive and allows the concurrent examination of populations of neurons.

In this paper, we demonstrate that $A\beta_{42}$ oligomer, but not monomer, produces significant reductions in neuronal spike activity of cultured neuronal networks. These network inhibitory effects appear to depend, either directly or indirectly, on modulation of both AMPA and NMDA mediated glutamate receptors. Moreover, two model compounds that have shown clinical promise in treating AD, methylene blue (MB) and memantine, appear to reverse or suppress the inhibitory effects of $A\beta_{42}$ oligomer. These observations suggest that cultured neuronal networks may be a potentially useful platform for screening therapeutic candidates for AD.

## 2. Results

### 2.1. *Stability of the synthesized $A\beta_{42}$ oligomer*

As shown in Fig. 1, the synthesized $A\beta_{42}$ oligomer in our work had a molecular weight of 4–85 kDa and showed high stability in saline buffer solution for at least 14 days at 4 °C and 25 °C (Duan et al., 2011; Matveeva et al., 2012). A slight shift in the oligomer size exclusion chromatography (SEC) profile was observed only after 14 days at 25 °C. Nevertheless, there was no evidence of fibrillization under the stability studies, as determined by SEC and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

### 2.2. *Extracellular recordings from in vitro cortical networks*

In total, 67 cultured neuronal networks *in vitro* derived from 11 mice were utilized in this study. As shown in Fig. 2A–C, cultured neurons on MEAs formed dense networks and became spontaneously active. The extracellular recordings were performed between days 21–32 *in vitro*. The overall percentage of the active electrodes or yield was 61±5% (mean ± SEM). Based on Novellino et al. (2011), any neuronal network with the yield below 25% was not considered sufficiently active for subsequent experiments. In the baseline recordings, the overall spike rate was 3.7±0.3 Hz and the number of bursts per minute was 7.3±0.3, while each burst had on average 11 spikes. All the reported measures were above the acceptance criteria for an active neuronal network (Novellino et al., 2011). The average spike amplitude in each unit was approximately 69±9 μV peak-to-peak. Consistent with prior work, the cortical networks showed synchronous bursting across a majority of the active channels. The signal-to-noise ratio (SNR) for a unit was defined as the mean peak-to-peak spike amplitude over the root mean square (RMS) value of the noise for the corresponding unit. The overall SNR for the baseline recordings was 11.2±0.2, suggesting the detection of well-resolved units from the neuronal networks on MEAs in this study.

### 2.3. *Functional responses to different forms of $A\beta_{42}$*

To compare the effects of $A\beta_{42}$ monomer and oligomer, the neuronal cultures were exposed at the concentration of 5 μM