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Multielectrode array analysis of cerebrospinal fluid in Alzheimer's disease versus mild cognitive impairment: A potential diagnostic and treatment biomarker

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ABSTRACT

Pathological cerebrospinal fluid (CSF) alterations like changes in amyloid-β_{1-42} and tau protein concentration are typical in Alzheimer's disease (AD). However, it remains unclear, if the composition of known or unknown pathological factors in native CSF has a functional significance in AD. In this pilot study, we used multielectrode array (MEA) neurochips to determine whether CSF of individuals with AD (AD-CSF) may have distinct neurofunctional properties that may distinguish it from that of individuals with mild cognitive impairment (MCI) – a differential diagnosis of high clinical importance. MEAs are neuronal cultures coupled to a multisite electrical recording system with the ability to reflect pharmacological or toxicological alterations on the functional level of whole neuronal networks. Collective rhythmical electrical activity was substantially enhanced after exposure to CSF of cognitively healthy subjects (controls) and of MCI individuals (MCI-CSF) alike. However, this activity increment was significantly reduced when MEAs were exposed to AD-CSF compared to MCI-CSF. Moreover, following AD-CSF exposure, networks showed significantly enhanced burst durations and less synchronous bursting, respectively. Thus, AD-CSF and MCI-CSF could be distinguished by characteristic changes of the network firing pattern on MEAs. When data of MCI individuals and AD patients were pooled, the network suppression correlated significantly with the degree of cognitive decline. The findings of this pilot study may set the stage for a unique and straightforward diagnostic bioassay of AD with particular value in the differential diagnosis to MCI and as a much needed biomarker for clinical trials.

1. Introduction

Based on the continuity between the neuronal extracellular environment and the cerebrospinal fluid (CSF), brain diseases frequently induce distinct alterations of the CSF composition that may directly reflect the pathological condition [1–3]. Such CSF alterations may themselves influence neuronal function, e.g., on the synaptic level, and thereby add to the pathophysiological process [4,5]. Their detailed analysis could provide insight into pathogenic mechanisms and foster the development of diagnostic and disease stage biomarkers which would be of particular value for research on neurodegenerative diseases and their treatment. In Alzheimer's dementia (AD), a severe neurodegenerative disorder with progressive and irreversible deterioration of cognitive and behavioral functions, CSF concentration shifts of the proteins amyloid-β_{1-42} ($A\beta_{1-42}$), total tau (t-tau), and phospho-tau 181 (p-tau), respectively, are well documented and have become part of the new diagnostic research criteria [6,7]. Moreover, on a functional level, in a bioassay using organotypic brain slices, CSF of AD patients (AD-CSF) has been demonstrated to exert neurotoxic properties [8]. However, none of these features of AD-CSF has so far been related to disease severity in the sense of a disease tracking marker [9].

In the present pilot study, we analyze the potential of multielectrode array (MEA) technology to possibly achieve that goal. Primary cortical neuronal networks grown on multielectrode neurochips develop synchronous oscillatory electrical activity that remains stable over weeks and even months and are sensitive to pharmacological and toxicological manipulations [10]. We [11–13] and others [14–17] have used MEA neurochip recordings as a powerful tool to rapidly and efficiently detect the neuroactive potency or neurotoxicity of various compounds. Network responses particularly to complex mixtures of neuroactive substances can

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be easily quantified in a multi-parametric manner, thus yielding 'fingerprints' of neuroactivity of the compounds in question [10]. The aim of our study was thus (1) to assess the system's response to AD-CSF exposure in general, (2) to determine whether this pattern of response may be characteristic and able to distinguish AD-CSF from CSF of MCI subjects (MCI-CSF), and (3) to correlate it to clinical disease severity.

2. Materials and methods

2.1. Subjects and procedures

We examined CSF samples from healthy controls and subjects with cognitive complaints who underwent diagnostic work-up in an academic outpatient memory clinic (Division of Geriatric Psychiatry, Department of Psychiatry and Psychotherapy, Heinrich Heine University, Düsseldorf). Diagnostic procedures included thorough physical, neurological, and psychiatric examination, routine blood chemistry, determination of CSF $A\beta_{1-42}$, t-tau and p-tau protein, respectively, as well as MRI brain scan and extensive neuropsychological testing. The latter included the Consortium to Establish a Registry of AD neuropsychological test (CERAD), the AD Assessment Scale – cognitive subscale (ADAS-cog), the Mini-Mental Status Examination (MMSE), and the Clock Drawing Test. Clinical diagnosis of probable AD was achieved according to NINCDS-ADRDA criteria and the new biomarker-based research criteria [7]. Diagnosis of MCI was established after Petersen [18].

2.2. CSF collection and processing

CSF samples from all subjects were obtained by lumbar puncture following informed consent and in accordance with a vote from the local Ethics Committee of the University of Düsseldorf/State of North Rhine-Westphalia, Germany. Sterile specimens were immediately centrifuged for 5 min at 1.850*g* and the supernatant was stored at −70 °C, avoiding repeated freeze/thaw cycles. Prior to analysis on MEA neurochips, CSF was thawed and the pH was adjusted to 7.4 with HEPES (4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid). In addition, standard CSF parameters were measured and the neurodegenerative CSF markers $A\beta_{1-42}$, t-tau, and p-tau protein were determined by commercial ELISA kits (Table 1).

2.3. Cell culture and electrophysiology

Neuronal networks were grown on MEA neurochips as described previously [19]. Briefly, suspensions of cryopreserved rat cortical neurons and glial cells (embryonic day 18) were purchased from CryoCell, QBM Cell Science, Ottawa, Canada, and stored in liquid nitrogen for up to 18 months. Before use, vials were thawed and diluted with prewarmed neurobasal medium supplemented with B27, glutamine, and penicillin–streptomycin (Invitrogen, Karlsruhe, Germany). Cells were plated at a density of $1.25 \times 105/cm^2$ on poly-D-lysine and laminin coated MEA dishes provided with a square grid of 60 planar Ti/TiN-microelectrodes (diameter: 30 μm, spacing: 200 μm) with an input impedance of <50 kΩ (MultiChannelSystems, Reutlingen, Germany). Cultures were then incubated in a humidified atmosphere (95% air, 5% CO_2) at 37 °C for 14–21 days. Spontaneous electrical activity was recorded as extracellular potentials directly from MEA dishes at 37 °C (head stage heating) for 2 min. Signals from individual electrodes were simultaneously sampled at 25 kHz, A/D-converted with 12 bits, visualized, and stored on hard disc. Offline spike and burst detection was performed by the custom-built software SpAnNer 1.0 (Tönisvorst, Germany).

Table 1
Sample characteristics.

Variable	MCI ($n = 7$)	AD ($n = 7$)	p^*
Age [yrs]	63.4 ± 2.6	69.5 ± 3.5	0.1825
Sex ratio (male/female)	2/5	3/4	1.0**
Cognitive test scores			
MMSE	25.9 ± 1.8	18.8 ± 3.1	0.0166
ADAS-cog	8.6 ± 2.1	20.0 ± 4.4	0.0419
BCRS	2.2 ± 0.4	4.1 ± 0.3	0.0211
WMS – working memory subtest	17.7 ± 3.7	6.8 ± 1.9	0.0571
TMT B/A	2.5 ± 0.4	4.2 ± 1.6	0.2061
CERAD subtests			
Verbal fluency	20.7 ± 1.7	14.0 ± 2.3	0.0431
Boston naming	13.8 ± 0.6	12.8 ± 1.0	0.1619
Word list – immediate recall	17.8 ± 2.6	10.0 ± 0.7	0.0539
Word list – delayed recall	4.8 ± 1.2	0.5 ± 0.3	0.0320
Constructional praxis	9.8 ± 0.6	8.2 ± 1.1	0.1112
Constructional praxis – recall	5.2 ± 1.3	0.8 ± 0.5	0.0160
CSF parameters			
$A\beta_{1-42}$ [pg/mL]	677.0 ± 91.8	413.3 ± 70.7	0.0111
t-tau [pg/mL]	366.6 ± 110.2	795.2 ± 126.7	0.0144
Total protein [mg/dL]	43.1 ± 3.6	30.6 ± 6.2	0.1508
Albumin [mg/dL]	26.1 ± 2.7	21.3 ± 2.3	0.2175
Q albumin [$\times 10^{-3}$]	6.1 ± 0.6	4.5 ± 0.6	0.0889
IgG [mg/dL]	2.7 ± 0.3	2.1 ± 0.4	0.4206
Q IgG [$\times 10^{-3}$]	2.8 ± 0.3	2.1 ± 0.7	0.1817

Abbreviations: ADAS-cog; Alzheimer's disease assessment scale – cognitive subscale, BCRS; brief cognitive rating scale, CERAD; consortium for the establishment of a registry for Alzheimer's disease, CSF; cerebrospinal fluid, Ig; immunoglobulin, MMSE; mini-mental status examination, Q; quotient, TMT; trail making test, WMS; wechsler memory scale.

* Mann–Whitney *U* test (if not otherwise indicated).

** Fisher's exact test.

2.4. Statistical analysis

Analyses were made in a multi-parametric manner and the following network parameters were extracted from raw spike train data: spike rate, burst rate, interburst interval, interspike interval within bursts, spike rate within bursts, and burst duration. To quantify the variation of activity patterns – reflecting possible changes in the spatiotemporal dynamics of the network activity – the standard deviations (SDs) of the aforementioned parameters were also determined. Statistical significance of clinical and experimental data was determined using unpaired *t* test or Mann–Whitney *U* test, as appropriate. Beforehand, the method of Kolmogorov and Smirnov was used to test for equal distribution of the data. Student's paired *t*-test was used to compare the spike rate before and after exposure of neurochips to CSF of control subjects. Spearman's nonparametric correlation test was used for correlation analysis. Statistical significance was set at $p < 0.05$. GraphPad Instat, version 3.0, was used for statistical analyses. Data are depicted as mean ± SEM throughout this paper.

3. Results

3.1. Demographic and clinical sample characteristics of MCI and AD subjects

Experimental data presented in this pilot report were obtained from CSF of 19 individuals, divided into three groups: control subjects ($n = 5$), subjects diagnosed with MCI ($n = 7$), and patients diagnosed with AD ($n = 7$). Basic demographic and clinical characteristics of both the MCI and AD sample, which were subjected to further analysis in our study, are listed in Table 1. Subjects did not differ significantly regarding mean age and sex distribution. Naturally, AD patients performed significantly worse in standard cognitive screening tests listed in Table 1. They also exhibited a typical

psychometric AD deficit profile and thus scored significantly lower in the verbal fluency and word list retrieval subtests of the CERAD battery, respectively, as well as on the working memory task of the Wechsler Memory Scale. The established CSF biomarkers $A\beta_{1-42}$, t-tau, and p-tau protein discriminated well between MCI and AD subjects with AD patients exhibiting low CSF $A\beta_{1-42}$ and high tau protein levels, defining the diagnosis. Other CSF parameters did not differ significantly and were within physiological limits.

3.2. Cortical cultures and spontaneous network activity

Neuritic outgrowth of cryopreserved dissociated embryonic rat neurons was visible under light microscopy already hours after plating. Within 1–2 weeks, a dense neuritic network was formed on the electrode layer of the MEAs (Fig. 1). By this time, a robust, stable, and synchronous spontaneous network activity was established. Mean baseline spike rate was 2867 ± 639/min. Cultures used in this study aged from 14 to 28 days *in vitro* (DIV).

3.3. Differential firing pattern in response to MCI-CSF versus AD-CSF

Application of pure buffered human CSF was well tolerated by rat primary cortical cells. Exposure to CSF of healthy control subjects resulted in a 2.8-fold increase of spontaneous spiking activity of the networks (Fig. 2). However, we found no statistically significant difference between spontaneous firing rate increments regarding CSF of control versus MCI-CSF (data not shown). By comparison, following administration of CSF of diagnosed mild to moderate AD patients (AD-CSF), the spike rate increase was significantly less pronounced (Fig. 3A). We subsequently carried out a multi-parametric analysis of the network dynamics in order to characterize the changes in overall activity state and intra- as well as interburst structure. The comparison between network responses to MCI- and AD-CSF regarding the most relevant network

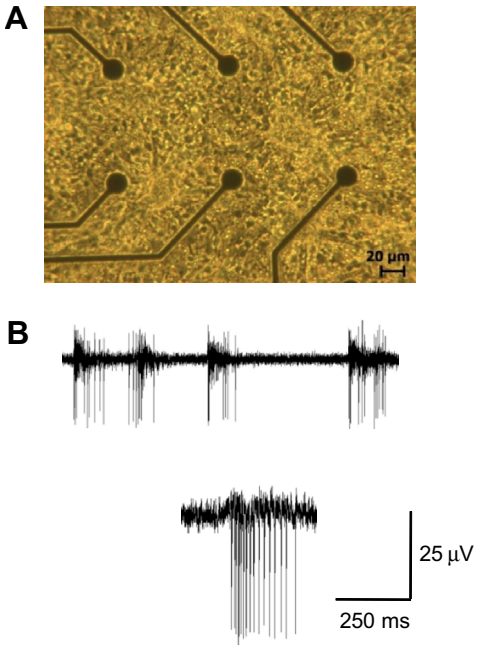

Fig. 1. (A) Matured neuronal network grown on a multielectrode array (MEA) after 7 days *in vitro* (DIV 7). A detailed view on a set of six out of 64 electrodes is shown with the dense cell layer attached to the MEA matrix (phase contrast microscopy). (B) Typical trace of spontaneous electrical activity from cortical neurons captured by a single electrode (above) and of an example burst consisting of 16 spikes (below). Spikes represent action potentials of single neurons in the vicinity of the electrode.

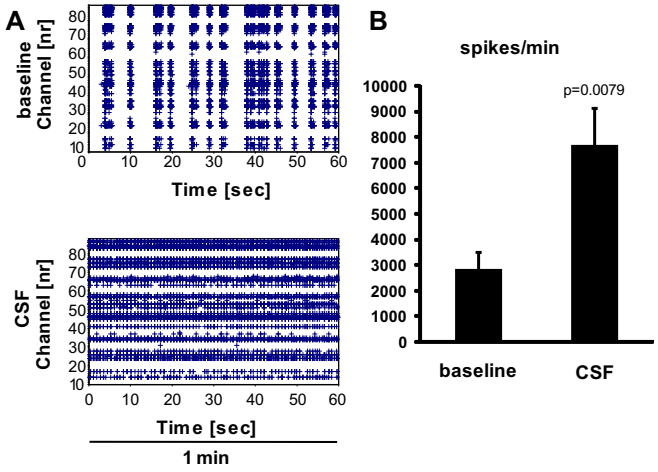

Fig. 2. Enhancement of neuronal network activity by human cerebrospinal fluid (CSF, healthy controls). (A) Spike raster plots show the spatiotemporal distribution of synchronized spontaneous neuronal network activity over all channels recorded within 1 min before (above) and after (below) exposure to CSF. (B) Overall network spike rate is enhanced by the factor 2.8 ($n = 5$).

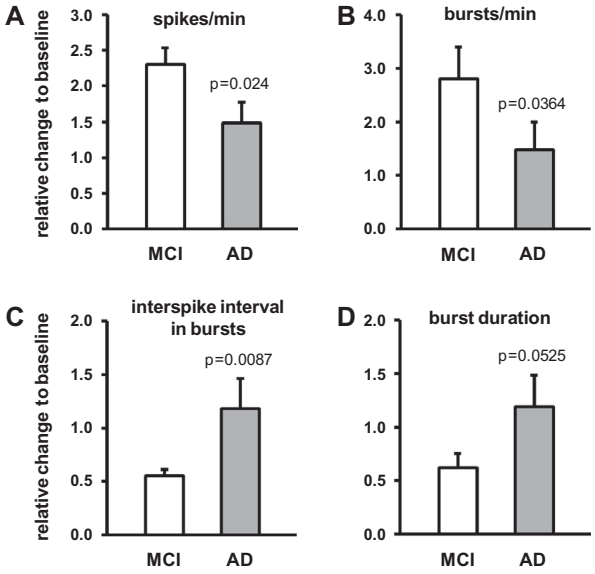

Fig. 3. Differential response of neuronal networks to cerebrospinal fluid (CSF) of individuals with mild cognitive impairment (MCI-CSF; $n = 7$) and Alzheimer's disease (AD-CSF; $n = 7$) as captured by salient network parameters (A–D).

parameters is depicted in Fig. 3. As illustrated, spontaneous spike and burst rate both increased only approx. 1.5-fold in response to AD-CSF compared to a 2.3- and 2.8-fold increase, respectively, upon exposure to MCI-CSF (Fig. 3A and B). Conversely, whereas MCI-CSF promoted a reduction of interspike intervals within bursts, AD-CSF mediated a slight increase and the difference between the two groups was highly significant (Fig. 3C). The burst duration showed a strong trend to increase with AD-CSF exposure compared to MCI-CSF (Fig. 3D). The remaining parameter displaying statistical significance between the two groups was the burst rate variance as assessed by the standard deviation of the number of bursts per minute. In both groups, the burst rate variance decreased upon CSF exposure – suggesting more regular activity – but less so with AD–CSF (0.5 versus 0.86, $p = 0.048$; not depicted). Other network parameters, as listed in Section 2, did not change significantly.

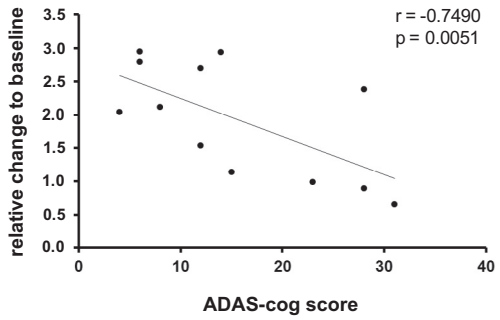

Fig. 4. Correlation of cerebrospinal fluid (CSF)-mediated enhancement of network activity (here: spike rate) and cognitive function in individuals with Alzheimer's disease (AD) and mild cognitive impairment (MCI) (pooled data). Reduced network response is associated with low cognitive status (high ADAS-cog score). $n = 12$ (Test data were not available for 2 subjects).

3.4. Neuronal network activity is correlated with cognition in MCI-CSF and AD-CSF

Next, we sought to analyze the relationship between global *in vitro* network activity after CSF exposure and cognitive function in all patients with cognitive complaints (i.e., MCI and AD). As illustrated in Fig. 4, reduced spike rate was significantly associated with the magnitude of cognitive decline as measured with the ADAS-cog scale – the most frequently used rating scale in clinical AD trials – and thus with disease severity. This led us to the conclusion that analysis of patient CSF on MEA neurochips may have the potential to serve as a surrogate biomarker.

4. Discussion

In earlier studies, we have investigated the influence of various neuroactive compounds on spontaneous neuronal network activity *in vitro*. In the present work, we confirmed the previous observation that exposure to human CSF on synchronously oscillating cultured networks derived from rat cortical cells was well tolerated and enhanced global activity parameters by a factor of 2–3 [4]. The cause of this network activity increase remains to be elucidated. With regard to the complex composition of human CSF, a variety of mechanisms are conceivable, including different electrolyte contents and the presence of CSF-specific components such as lactate and excitatory amino acids (EAAs) which are contained in the physiological CSF [20]. As an example, glutamate has been shown to promote a lasting potentiation of synaptic transmission in cultured hippocampal neurons [21]. We exclude an influence of the pH value since in our study, pH values of all CSF specimens were adjusted to 7.4 as in the original culture medium immediately before application.

In this pilot study, global network activity change upon CSF application was not different between healthy controls and individuals with MCI. However, we found a significant difference regarding the neuronal firing pattern when the network response to MCI-CSF was compared to AD-CSF. The latter promoted an attenuated net activity increase and longer bursts. Moreover, the significantly higher interburst interval's variability in AD-CSF indicates a relative decline of network synchrony. Therefore, the cultured network can be viewed as a biosensor to AD-CSF that changes its spatiotemporal characteristics in a distinct manner. It does so most likely in response to a specific single or combination of inhibitory neuroactive components of the AD-CSF, probably via interfering with one or more neuronal receptors or ion channels. The inhibitory factor(s) may be directly secreted into the extracellular fluid and CSF by neuronal or glial cells or stem from a peripheral source after crossing the blood brain barrier (BBB). As a matter of fact, in AD, dysfunction of the BBB regularly occurs from very early stages on and may be an integral part of the disease's pathophysiology [22]. As mentioned above, a previous report of neurotoxic properties of AD-CSF exists, but with no association to disease severity [8]. At this point, the identity of the AD-CSF constituent that disturbs network function in our system remains a matter of speculation. The presence of $A\beta_{1-42}$ oligomers – which are increasingly recognized as a key factor in AD pathogenesis – in AD-CSF may play a role since we have demonstrated a dose-dependent network suppression of $A\beta_{1-42}$ in a previous study [11]. Moreover, corresponding to the correlation with network suppression observed in the present study, recent observations point to a similar correlation of AD-CSF $A\beta_{1-42}$ oligomer counts with cognitive decline [23,24]. Alternatively, excitotoxic substances that may trigger intracellular Ca^{2+} influx could be suspected or reactive oxygen species or high levels of inflammatory mediators, as such factors are also believed to contribute substantially to pathogenesis and course of the disease.

The data of our pilot study suggest that MEA neurochips may detect distinct neurofunctional properties of AD-CSF compared to MCI-CSF. Most importantly, the observed changes correlated with the degree of cognitive decline and thus with dementia severity. These findings may set the stage for a new and straightforward diagnostic and, above all, disease tracking biomarker which has not yet been developed but is much needed to monitor efficacy in clinical trials. The small sample size of our study, which was designed as a pilot project, is of course a limitation. A strength is, however, the high diagnostic accuracy of AD and MCI because the diagnosis was not only based on clinical, neuroimaging, and neuropsychological grounds but also on established chemical biomarker status according to the latest biomarker-aided diagnostic criteria of AD [6]. In follow-up studies with large numbers of AD, MCI, and control subjects, respectively, we plan to further validate the relationships reported here, particularly regarding the suitability of this system as a surrogate biomarker in therapeutic trials. Further research is also needed to identify the substance(s) and pathway(s) that mediate the observed relative suppression of *in vitro* network activity in AD-CSF.

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References

[1] H. Tumani, J. Brettschneider, Biochemical markers of autoimmune diseases of the nervous system, Curr. Pharm. Des. 18 (2012) 4556–4563.
[2] J. Tarasiuk, A. Kulakowska, W. Drozdowski, J. Kornhuber, P. Lewczuk, CSF markers in amyotrophic lateral sclerosis, J. Neural Transm. 119 (2012) 747–757.
[3] A. Stefani, L. Brusa, E. Olivola, M. Pierantozzi, A. Martorana, CSF and clinical hallmarks of subcortical dementias: focus on DLB and PDD, J. Neural Transm. 119 (2012) 861–875.
[4] F. Otto, S. Illes, J. Opatz, M. Laryea, S. Theiss, H.P. Hartung, A. Schnitzler, M. Siebler, M. Dihne, Cerebrospinal fluid of brain trauma patients inhibits in vitro neuronal network function via NMDA receptors, Ann. Neurol. 66 (2009) 546–555.
[5] N.J. Abbott, Evidence for bulk flow of brain interstitial fluid: significance for physiology and pathology, Neurochem. Int. 45 (2004) 545–552.
[6] B. Dubois, H.H. Feldman, C. Jacova, J.L. Cummings, S.T. DeKosky, P. Barberger-Gateau, A. Delacourte, G. Frisoni, N.C. Fox, D. Galasko, S. Gauthier, H. Hampel, G.A. Jicha, K. Meguro, J. O'Brien, F. Pasquier, P. Robert, M. Rossor, S. Salloway, M. Sarazin, L.C. de Souza, Y. Stern, P.J. Visser, P. Scheltens, Revising the definition of Alzheimer's disease: a new lexicon, Lancet Neurol. 9 (2010) 1118–1127.
[7] S. Gauthier, B. Dubois, H. Feldman, P. Scheltens, Revised research diagnostic criteria for Alzheimer's disease, Lancet Neurol. 7 (2008) 668–670.

[8] J. Marksteiner, M. Pirchl, C. Ullrich, H. Oberbauer, I. Blasko, W. Lederer, H. Hinterhuber, C. Humpel, Analysis of cerebrospinal fluid of Alzheimer patients, Biomarkers and toxic properties, Pharmacology 82 (2008) 214–220.
[9] H. Hampel, Y. Shen, D.M. Walsh, P. Aisen, L.M. Shaw, H. Zetterberg, J.Q. Trojanowski, K. Blennow, Biological markers of amyloid beta-related mechanisms in Alzheimer's disease, Exp. Neurol. 223 (2010) 334–346.
[10] A.F. Johnstone, G.W. Gross, D.G. Weiss, O.H. Schroeder, A. Gramowski, T.J. Shafer, Microelectrode arrays: a physiologically based neurotoxicity testing platform for the 21st century, Neurotoxicology 31 (2010) 331–350.
[11] P. Görtz, J. Opatz, M. Siebler, S.A. Funke, D. Willbold, C. Lange-Asschenfeldt, Transient reduction of spontaneous neuronal network activity by sublethal amyloid beta (1–42) peptide concentrations, J. Neural Transm. 116 (2009) 351–355.
[12] P. Görtz, A. Hoinkes, W. Fleischer, F. Otto, B. Schwahn, U. Wendel, M. Siebler, Implications for hyperhomocysteinemia: not homocysteine but its oxidized forms strongly inhibit neuronal network activity, J. Neurol. Sci. 218 (2004) 109–114.
[13] P. Görtz, H. Koller, B. Schwahn, U. Wendel, M. Siebler, Disturbance of cultured rat neuronal network activity depends on concentration and ratio of leucine and alpha-ketoisocaproate: implication for acute encephalopathy of maple syrup urine disease, Pediatr. Res. 53 (2003) 320–324.
[14] A. Gramowski, K. Jugelt, S. Stuwe, R. Schulze, G.P. McGregor, A. Wartenberg-Demand, J. Loock, O. Schröder, D.G. Weiss, Functional screening of traditional antidepressants with primary cortical neuronal networks grown on multielectrode neurochips, Eur. J. Neurosci. 24 (2006) 455–465.
[15] Y. Xia, K.V. Gopal, G.W. Gross, Differential acute effects of fluoxetine on frontal and auditory cortex networks in vitro, Brain Res. 973 (2003) 151–160.
[16] G. Xiang, L. Pan, L. Huang, Z. Yu, X. Song, J. Cheng, W. Xing, Y. Zhou, Microelectrode array-based system for neuropharmacological applications with cortical neurons cultured in vitro, Biosens. Bioelectron. 22 (2007) 2478–2484.
[17] Y. Xia, G.W. Gross, Histiotypic electrophysiological responses of cultured neuronal networks to ethanol, Alcohol 30 (2003) 167–174.
[18] R.C. Petersen, Mild cognitive impairment as a diagnostic entity, J. Intern. Med. 256 (2004) 183–194.
[19] F. Otto, P. Görtz, W. Fleischer, M. Siebler, Cryopreserved rat cortical cells develop functional neuronal networks on microelectrode arrays, J. Neurosci. Methods 128 (2003) 173–181.
[20] X. Mu, A. Wu, J. Wu, Y. Liu, Y. Zhang, Y. Yue, L. Fang, Y. Wang, Effects of anesthetic propofol on release of amino acids from the spinal cord during visceral pain, Neurosci. Lett. 484 (2010) 206–209.
[21] I. Antonova, O. Arancio, A.C. Trillat, H.G. Wang, L. Zablow, H. Udo, E.R. Kandel, R.D. Hawkins, Rapid increase in clusters of presynaptic proteins at onset of long-lasting potentiation, Science 294 (2001) 1547–1550.
[22] B.V. Zlokovic, Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders, Nat. Rev. Neurosci. 12 (2011) 723–738.
[23] L. Wang-Dietrich, S.A. Funke, K. Kuhbach, K. Wang, A. Besmehn, S. Willbold, Y. Cinar, O. Bannach, E. Birkmann, D. Willbold, The Amyloid-beta oligomer count in cerebrospinal fluid is a biomarker for Alzheimer's disease, J. Alzheimers Dis. 34 (2013) 985–994.
[24] A.N. Santos, M. Ewers, L. Minthon, A. Simm, R.E. Silber, K. Blennow, D. Prvulovic, O. Hansson, H. Hampel, Amyloid-beta oligomers in cerebrospinal fluid are associated with cognitive decline in patients with Alzheimer's disease, J. Alzheimers Dis. 29 (2012) 171–176.