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EVENT ABSTRACT

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Neuronal Networks Coupled To Microelectrode Arrays: Network Maturation Impairments In Neurodevelopmental Disorders

Monica Frega[1*], Jason Keller[1], Britt Mossink[1], Katrin Linda[2], Bas Van Gestel[2], Nael N. Kasri[1] and Dirk Schubert[3]

[1] Radboudumc, Department of Cognitive Neurosciences, The Netherlands
[2] Radboud University, Donders Institute for Brain Cognition and Behaviour, The Netherlands
[3] University Medical Centre Nijmegen, The Netherlands

Motivation
Neurodevelopmental disorders (NDDs), including intellectual disability (ID) and autism spectrum disorders, are phenotypically and genotypically heterogeneous. One increasing group of NDDs is caused by mutations in epigenetic regulators of gene expression. Recent studies have identified Euchromatin Histone Methyltransferase 1 (EHMT1) as a key modulator in cognition. Heterozygous loss of EHMT1 causes Kleefstra syndrome, which is characterized by moderate-to-severe ID, autistic behaviour, microcephaly and dysmorphic features [1].
While the identification of disease genes has great benefits for diagnostic and prognostic purposes, for the vast majority of these genes there is little knowledge about neurobiological mechanisms that they control at the cellular and network level. Recent studies demonstrate that NDDs are "diseases of the synapse" [2]. Synaptic malfunction can severely affect network connectivity and dynamic. Understanding the neural circuit basis of NDDs is therefore imperial for a better understanding of these disorders.
Here, we studied the effect of EHMT1-deficiency on neuronal activity in-vitro. To understand whether the disruption of EHMT1 gives rise to an impairment of the network maturation, we monitored the electrophysiological activity of neuronal networks coupled to micro-electrode arrays (MEAs) during network formation and maturation. We used three models: (1) rat dissociated cortical neurons; (2) dissociated neuronal cultures from a heterozygous knock-down mouse; and (3) neurons derived from Induced Pluripotent Stem Cells (iPSC) of Kleefstra patients.

Material and Methods
Mouse and rat dissociated neuronal cultures. Dissociated neuronal cultures were obtained from cortex of embryonic mice and rats and plated onto MEAs (Multi Channel Systems - MCS, Reutlingen, Germany) at a final density of 1200 cells/mm2. At Day In Vitro 2 (DIV) the cells dissociated from rat were transduced with lentivirus containing a GFP gene and a shRNA targeting mRNA of EHMT1.

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into upper-layer cortical neurons by overexpressing the neuronal determinant neurogenin 2 (NGN2) upon doxycycline treatment [3]. The derived neurons were plated onto MEAs at a final density of 1200 cells/mm2.
Statistics. Data are expressed as mean ± standard error of the mean. Statistical analysis were performed with Mann–Whitney test.

Results
In order to understand whether the disruption of EHMT1 gives rise to an impairment of the network maturation we monitored the electrophysiological activity of a culture from wild-type (WT, n=12) and Ehmt1+/- mice (n=10) on MEAs during development. Figure 1 shows raster plots of the spontaneous activity of a representative experiment performed on WT and EHMT1-deficient networks. EHMT1-deficient network presented less global activity and synchrony compared to the WT condition; furthermore the regularity of the network busting activity was impaired.
During early development the level of firing rate of the EHMT1-deficient network was significantly lower ($p<0.01$) than the one exhibited by the control network at DIV 13 (WT 1.1±0.1 spike/s; Ehmt1 0.7±0.1 spike/s), DIV 15 (WT 2.1±0.2 spike/s; Ehmt1 1.1±0.2 spike/s) and DIV 17 (WT 2.1±0.2 spike/s; Ehmt1 1.2±0.2 spike/s). Furthermore the rate of synchronous events generated by the EHMT1-deficient networks was significantly lower (DIV 13, WT 4.3±0.9 burst/min; Ehmt1 1.1±0.4 burst/min. DIV 15, WT 12.9±1.8 burst/min; Ehmt1 1.7±0.6 burst/min. DIV 17, WT 7.5±1.1 burst/min; Ehmt1 1.6±0.6 burst/min). Later in development (i.e. DIV 20) the EHMT1-deficient network showed a level of activity similar to the control (WT, 2.0±0.2 spike/s; Ehmt1, 1.6±0.2 spike/s),however the synchronized bursting activity remained impaired by means of significantly different bursting rates (WT 8.2±1.1 burst/min; Ehmt1 2.6±0.7 burst/min) and in a lower level of network regularity (i.e. coefficient of variability computed on the interval between two consecutive network burst is statistically higher in the EHMT1-deficient networks).

In a next step we recorded the electrophysiological activity of dissociated neuronal cultures obtained from rats in which EHMT1 expression was knocked-down (WT, n=16 and Ehmt1, n=13). Our data showed that induced EHMT1 deficiency affected network maturation at a level that was comparable with that of neuronal networks from heterozygous mice.

In a third set of experiments we recorded the electrophysiological network activity of human neurons derived from healthy subjects and Kleefstra patients. Few days after plating on MEAs, the neurons derived from healthy subject formed functionally active neuronal networks, showing spontaneous events already during the second week in vitro. Late in development (i.e. fourth week in vitro) the neuronal network showed high level of spontaneous activity as well as synchronous network bursts. Neurons derived from Kleefstra patients established spiking activity during early network development as well as synchronous events involving most of the channels of the MEAs later in development, too. However, our preliminary results imply a difference between the two genetic conditions in terms of connectivity and network organization.

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Our data show that the emergence of spontaneous network activity was delayed in EHMT1-deficient networks compared to control condition. The delay in spontaneous network activity early in development resulted in an increased network burst irregularity in later time points. These results support the notion that early developmental deficits could lead to irreversible changes in network wiring and synchronization. This suggests that EHMT1 may play a critical role in a developmental mechanism of gene expression regulation during early brain development.

Conclusion

Our results show that epigenetic regulation by EHMT1 is important for the proper development of neuronal circuits. The identification of reasons that cause abnormal network activity will be important for the understanding, and ultimately treatment, of Kleefstra syndrome. Furthermore as our models produce reliable functional data on disease related network formation and maturation both on animal as well as human material, it will allow us to perform disease specific pharmacological and genetic rescue experiments.

References

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Figure Legend

Figure 1. Raster plot showing 1 min of spontaneous activity of neuronal networks at DIV 15. Red boxes highlight network burst.

Figure 1

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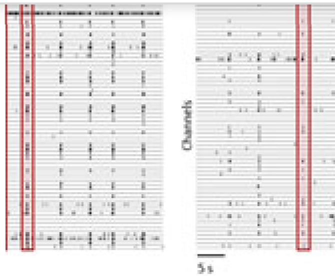

Keywords: Human Induced Pluripotent Stem Cells, neurodevelopmental disorder, activity impairments, derived neurons

Conference: MEA Meeting 2016 | 10th International Meeting on Substrate-Integrated Electrode Arrays, Reutlingen, Germany, 28 Jun - 1 Jul, 2016. **Presentation Type:** oral

Topic: MEA Meeting 2016

Citation: Frega M, Keller J, Mossink B, Linda K, Van Gestel B, Kasri NN and Schubert D (2016). Neuronal Networks Coupled To Microelectrode Arrays: Network Maturation Impairments In Neurodevelopmental Disorders. *Front. Neurosci. Conference Abstract: MEA Meeting 2016 | 10th International Meeting on Substrate-Integrated Electrode Arrays*. doi: 10.3389/conf.fnins.2016.93.00001

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Received: 22 Jun 2016; **Published Online:** 24 Jun 2016.

*** Correspondence:** Dr. Monica Frega, Radboudumc, Department of Cognitive Neurosciences, Nijmegen, The Netherlands, m.frega@utwente.nl

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