Increased excitability in hippocampal neurons of synaptopodin-knockout mouse

Etay Aloni
Technion Israel Institute of Technology
Serphima Verbitzky
Weizmann Institute of Science Faculty of Biology
Lilia kushnireva
Perm State University: Permskij gosudarstvennyj nacional'nyj issledovatel'skij universitet
Eduard Korkotian
Weizmann Institute of Science Faculty of Biology
Menahem Segal (menahem.segal@weizmann.ac.il)
Weizmann Institute of Science https://orcid.org/0000-0001-7592-0408

Short Report

Keywords: synaptopodin, dendritic spines, hippocampus, excitability

Posted Date: May 13th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-494756/v1

Version of Record: A version of this preprint was published at Brain Structure and Function on July 21st, 2021. See the published version at https://doi.org/10.1007/s00429-021-02346-0.

BSAF
May 3, 2021

Increased excitability in hippocampal neurons of synaptopodin-knockout mouse

Aloni E., Verbitsky S., Kushnireva L., Korkotian E. and Segal M.[1]

[1] Corresponding author

Abbreviated title: hippocampus in SPKO mice

Department of Neurobiology, Weizmann Institute, Rehovot 76100 Israel
e-mail address: menahem.segal@weizmann.ac.il *Orcid ID Number:*0000-0001-7592-0408

DECLERATIONS:
Conflict of interest: The authors declare no competing financial interests.
Contributions: All co-authors agree to have their names listed as authors.
Acknowledgements: We would like to thank Drs. R. Eilam and S. Haidarliu for help with the immunohistochemical analysis.
Funding: Supported by a grant from the Clore Center for Biological Physics of the Weizmann Institute of Science.
Ethical Approval: Experiments were conducted by the rules of the Institutional Animal Care and use Committee, approval number: 38390917-2
Author contributions: EA conducted and analyzed the extracellular electrophysiological experiments, and wrote the manuscript. SV conducted and analyzed the patch recording experiments, LK conducted the behavioral and immunohistochemical studies, EK and MS wrote the manuscript. All co-authors have read and commented on the manuscript and agreed to have their names listed as authors.

Keywords: synaptopodin, dendritic spines, hippocampus, excitability

Abstract

Synaptopodin (SP) is localized within the spine apparatus, an enigmatic structure located in the neck of spines of central excitatory neurons. It serves as a link between the spine head, where the synapse is located, and the endoplasmic reticulum (ER) in the parent dendrite (Vlachos et al. 2009, Korkotian and Segal, 2011, Zhang et al. 2013). SP is also located in the axon initial segment, in association with the cisternal organelle, another structure related to endoplasmic reticulum. Extensive research using SP knockout (SPKO) mice suggests that SP has a pivotal role in structural and functional plasticity (Deller et al. 2003, Deller et al. 2007). Consequently, SPKO mice were shown to be deficient in cognitive functions, and in ability to undergo long term potentiation of reactivity to afferent stimulation (Deller et al. 2003). In contrast, neurons of SPKO mice appear to be more excitable than their *wild type* (wt) counterparts(Bas Orth et al, 2007). To address this discrepancy, we have now recorded activity of CA1 neurons in the mouse hippocampus slice, with both extracellular and patch recording methods. Electrophysiologically, SPKO cells in CA1 region of the dorsal hippocampus were more excitable than wt ones. In addition, exposure of mice to a complex environment caused a higher proportion of arc-expressing cells in SPKO than in wt mice hippocampus. These experiments indicate that higher excitability and higher expression of arc staining may reflect SP deficiency in the hippocampus of adult SPKO mice.

INTRODUCTION

Synaptopodin (SP) is an actin-associated protein localized in the neck of dendritic spines of mature cortical and hippocampal neurons, in physical association with the enigmatic spine apparatus (Aloni et al. 2019, Mundel et al. 1997, Deller et al. 2000, Segal et al. 2010). It is also found in the cisternal organelle of the axon initial segment (Segal 2018). These are two strategic locations, where SP is associated with calcium stores of the endoplasmic reticulum (ER) and can control the inputs and outputs of the neurons. Recent studies have assigned a role for SP in synaptic plasticity (Vlachos et al 2009), and assumed that it links morphological changes in actin cytoskeleton with functional synaptic changes generated in response to plasticity-producing stimulation. Studies conducted with SP-knockout mice (SPKO) (Deller et al. 2003), found that these mice are deficient in cognitive tasks and that slices taken from SPKO mice are deficient in ability to generate long term plasticity. Later studies proposed that only some LTP generating protocols are sensitive to the absence of SP (Jedlicka and Deller 2017). Concerning the role of SP in spine plasticity, it has been shown that overexpression of (transfected) SP maintains the activity-dependent spine enlargement (Okubo-Suzuki et al. 2008), and that SP-positive spines are more amenable to plastic changes, which are associated with activation of calcium stores (Harris, 1999). However, most of these studies were conducted with young mice, leaving the issue of what changes in the SPKO mice when they grow up, to enable plasticity, unanswered. In the present study we explored some molecular and cellular mechanisms in the adult SPKO mice, and would like to report that these mice express behavior-induced higher arc activation

than wt mice and that neurons in SPKO mice are more excitable than those of wt controls. These mechanisms may counterbalance the lack of SP in the adult mouse.

METHODS

Animals:

Experiments were conducted by the rules of the Institutional Animal Care and use Committee. Forty-four male mice, aged 6-8 months were used for all of the experiments. Mice were maintained on a 12 h light/dark cycle, and were allowed free access to food and water. Since in earlier studies there was no apparent difference between wt and heterozygous (hetero), in the behavioral experiments the results of the two groups were merged (see below).

Behavioral experiments:

Animals were divided into 2x2 groups: (1) wt/hetero and SPKO mice that were sacrificed directly out of their home cages (control, Ctrl); (2) wt/hetero and SPKO mice that explored a new open field environment for 5 minutes twice, separated by 20 min. The open field was a square box (30 × 30 cm) with 15cm high walls. A standard small (8cm) rotating disk was placed in the middle of the box. In each exploration session, mice were lifted and randomly placed in the box. Fifty minutes after the first exposure to the open field, mice were sacrificed. All mice explored the space and ran in the rotating disk, but their behavior was apparently not different between the two groups, and was not quantified.

Imaging and analysis

Confocal image stacks were taken using a Zeiss LSM 880 laser scanning microscope equipped with EC plan-Neofluar x5/0.16 M27, plan-apochromat 20x/0.8 and plan-apochromat 63x/1.40 oil DIC objectives. Detector and amplifier gain were initially set to obtain pixel densities within a linear range. Eight image stacks were recorded for each hippocampus. Arc-positive and c-Fos -positive cells were counted from each field size of 135x135 µm (63x/1.40 oil DIC objectives). Cell count and fluorescence levels were measure using Image-J software. Measurements were made in a double-blind procedure by an independent observer to assure unbiased analysis. Statistical comparisons were made using Origin software.

Extracellular Electrophysiology

Mice were rapidly decapitated with a guillotine, their brain removed and the hippocampus was sliced into transverse 400 µm slices on a McIllwain tissue chopper. Slices were incubated at room temperature for 1.5 h in carbogenated (5% CO2 / 95% O2) ACSF (124 mM NaCl, 4.2 mM KCl, 26 mM NaHCO3, 1.24 mM KH2PO4, 2.5 mM CaCl2, 2 mM MgSO4 and 10 mM glucose, at pH=7.4). Recordings were made from interface slices in a standard chamber at 33.8–34.0°C. Field excitatory postsynaptic potentials (EPSPs) were recorded through a glass pipette containing 0.75 M NaCl (4 MΩ) in stratum radiatum of CA1 region. Synaptic responses were evoked by stimulation of the Schaffer collaterals through bipolar handmade

Nickel-Chromium electrode. Two stimulating electrodes were located on both sides of the recording electrode, with both stimulating the schaffer collateral pathway. Data acquisition and off-line analysis were performed using pCLAMP 9.2 (Axon Instruments) in a blind procedure.

Whole cell patch recordings:

Mice were rapidly decapitated with a guillotine, their brain removed and sliced using vibratome into transverse 350 µm slices in 4°C oxygenated (5% CO2/ 95% O2) sucrose solution (in mM: 2.5 KCl, 26 NaHCO3, 1.25 NaHPO4, 10 glucose, 10 MgSO4, 0.5 CaCl and 234 sucrose). Slices were incubated at 37°C for 45 minutes, after which they were transferred and recorded at room temperature in carbogenated (5% CO2 / 95% O2) ACSF as above. CA1 pyramidal cells were recorded with patch pipettes containing (in mM) 136 K-gluconate, 10 KCl, 5 NaCl, 10 HEPES, 0.1 EGTA, 0.3 Na-GTP, 1 Mg-ATP, and 5 phosphocreatine, pH 7.2 (with a resistance of 5–10 MΩ). 1% biocytin was added to the intracellular solution for later morphological assessment. Current pulses of 800ms were injected to assess cell's passive properties and spiking behavior (Fig 2), first pulse was -200pA, increment 100pA. Spike characteristics and interspike intervals were analyzed from the first five current injections that evoked more than 2 spikes. Signals were amplified with Axopatch 200B and recorded with PClamp-10 (Axon Instruments). Data were analyzed using Matlab R2015b, MiniAnalysis and Microsoft Excel.

Action potential kinetics analysis:

Current-clamp recordings were imported in Matlab where the first ten action potentials (AP) that did not arrive in bursts or too close to the end of the current pulse where collected with 5ms pre-peak and 65ms post-peak; these spikes were aligned at peak, averaged and this average was used to calculate a phase plot. The average and standard error were calculated for these phase plots within every group. Numeric voltage derivative was calculated as difference between the voltages recorded at neighboring sampling points; multiplied by sampling rate (per ms) when appropriate. AP onset was calculated as a time-point where voltage derivative exceeds maximum numerical voltage derivative at 2.5ms of 5ms before the spike multiplied by 1.5. AP threshold was calculated as voltage at AP onset. AP shape characteristics were calculated individually for each of the 10 APs per cell; these characteristics where averaged for every cell. AP amplitude and AP after-hyperpolarization were calculated relative to AP threshold. Half-width was calculated as difference between time points where voltage reached (AP peak+ AP threshold)/2 at rise and at decay. Rise and decay slopes were calculated as maximal and minimal numeric voltage derivative, respectively.

Statistical analysis: All experiments were analyzed using unpaired Student's t tests or ANOVA, as the case may be. Results are expressed as mean±SEM, Statistical significance was set at $p<0.05$.

RESULTS

A: Electrophysiological properties of SPKO hippocampus: extracellular recording

The higher elevation of arc in active SPKO mice compared to wt suggests a higher excitability of hippocampal neurons. To test this directly, experiments with hippocampal slices were conducted. First, population EPSPs in field recording were measured at three different stimulation intensities. CA1 cells produced significantly larger population EPSPs in SPKO compared to wt (Fig 1A). Furthermore, SPKO slices produced population spikes at lower stimulation intensities than wt slices. In addition, Paired pulse facilitation was measured at three different inter-pulse intervals (IPI). We found a trend of higher paired pulses facilitation in the SPKO group compared to wt mice, in all three IPI's tested. However, there was no statistically significant difference between the two groups (Fig 1B).

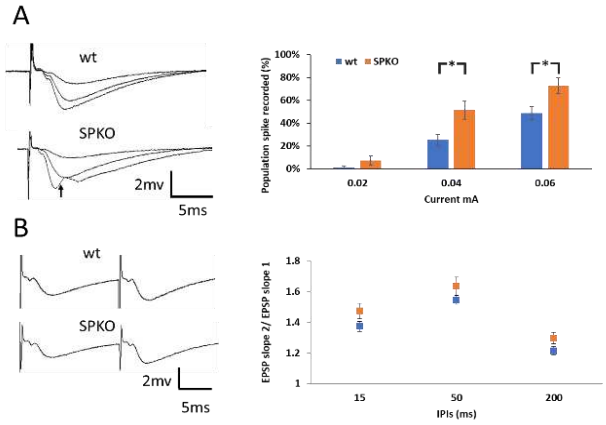

Figure 1. Higher excitability in SPKO CA1 pyramidal neurons. Field potential recordings in the Schaffer collaterals of the hippocampus of 6-month-old mice A. Left: sample population EPSP responses to three stimulation intensities in wt and SPKO mice. Black arrow points at population spike recorded in SPKO mice in response to a pulse of 0.06mA stimulation. Right: Averaged population responses to increasing stimulation of stratum radiatum in wt (n=39 slices, 11 mice) and SPKO (n=20 slices, 4 mice). (0.02mA: X^2_1=3.122, p=0.077, 0.04mA: X^2_1=8.413, p=0.004, 0.06mA: X^2_1=6.636, p=0.01). B. Left: samples of population EPSP responses to paired pulse stimulation at three inter pulse intervals (IPI) in wt and SPKO mice. Right: Averaged Paired pulse facilitation in three different IPI's in wt (n=28 slices, 7 mice) and SPKO (n= 17 slices 4, mice). (15ms IPI: F_{43}= 0.425, p=0.086, 50ms IPI: F_{43}= 2.648, p=0.12, 200ms IPI: F_{43}= 0.384, p=0.072).

B: Electrophysiological properties of SPKO hippocampus: intracellular recording

Using patch clamp recording in hippocampal slice, no differences in passive properties were found between SPKO and wt neurons (resting membrane potential, membrane time constant, input resistance), nor did they differ in action potential (AP) threshold or the numbers of APs produced per current pulse (data not shown). However, the AP's in SPKO cells had markedly different kinetics; in particular, AP's elicited by SPKO neurons were larger in amplitude, shorter in width (Fig. 2A, D), had smaller after-hyperpolarization (AHP) and a steeper rise (Fig. 2B, D).

In another series of experiments, CA1 neurons were voltage clamped at -70mV, and synaptic currents were recorded under standard conditions. No synaptic blockers or intracellular anesthetics were used. The spontaneous events represent a mix of excitatory and inhibitory currents. The overall frequency of these events was about 50% higher in slices obtained from SPKO mice compared to wt controls (Fig 2C, E).

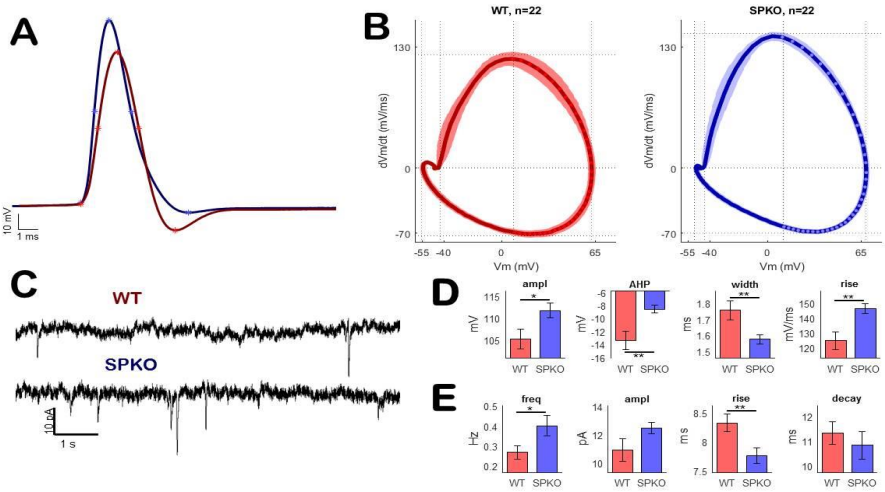

Figure 2. Properties of patch-clamped CA1 neurons in 6-month-old wt and SPKO mice. **A.** Sample plots showing averages of 10 AP recorded from a WT cell (red) and a SPKO cell (blue), aligned at AP threshold. Asterisks denote AP threshold (see methods), AP half-amplitude, AP peak and AP after-hyperpolarization. **B**. Phase plots showing differences in AP kinetics. For every cell 10 AP were selected and phase plot were averaged. Solid line presents averages of AP phase plots constructed from all cells recorded within a group; shadow shows SEM's calculated from deviations of every phase plot from the mean. Vertical dotted lines show AP afterhyperpolarization (AHP) current, AP threshold, AP half-width and AP amplitude (left to right). Horizontal dotted lines show AP rise slope, origin and AP decay slope (from top to bottom). Colored dots show digitizer sampling frequency within an AP recording. **C.** Sample recording of spontaneous synaptic activity. Scale bars: 10pA, 1s. **D**. Bar graphs showing properties of AP kinetics in the two groups, left to right: AP amplitude (peak-threshold), WT 105.4±2.2 mV, SPKO 111.9±1.7 mV, $p<0.03$; AP AHP (after-hyperpolarization, after-spike minimum – threshold), WT -13.3±1.4 mV, SPKO -8.5±0.6 mV, $p<0.005$; AP width (measured at half-amplitude – midpoint between AP threshold and AP peak), WT 1.76±0.06 ms, SPKO 1.58±0.03 ms, $p<0.01$; AP maximal rise slope, WT 125.4±5.9 mV/ms, SPKO 146.7±3.2 mV/ms, $p<0.005$. AP threshold (not shown) did not differ significantly between the groups; WT -42.7±0.8 mV, SPKO -43.3±1; neither did differ AP maximum decay slope, WT -75.1±2.9 mV/ms, SPKO -71.8±1.7 mV/ms. **E**. Bar graphs showing differences in spontaneous postsynaptic current properties between the groups. Obtained through patch-clamp recordings at -70mV, no synaptic blockers present. Each recording was >2min/cell, 22 cells WT, 19 cells SPKO. Left to right: sPSC frequency WT 0.27±0.03 Hz, SPKO 0.4±0.05 Hz, $p<0.03$; sPSC amplitude WT 10.9±0.8 pA, SPKO 12.5±0.4 pA, n.s.; sPSC rise time WT 8.3±0.2 ms, SPKO 7.8±0.1 ms, $p<0.01$; sPSC decay time WT 11.4±0.4 ms, SPKO 10.9±0.6 ms, n.s. All data presented in mean±SEM, p-values measured using unpaired Student t-test.

C: Activation of arc and cFos in active wt and SPKO mice

As arc and cFos are activity-dependent genes, their expression in the dentate gyrus of the dorsal hippocampus was examined by IHC in wt and SPKO mice after exposure to new environment. The active mice expressed significantly higher number of arc neurons in the SPKO than the wt mice (Fig 3A-C. cFos expression was also elevated in the active mice, but did not significantly differ between the two groups (Fig 3D).

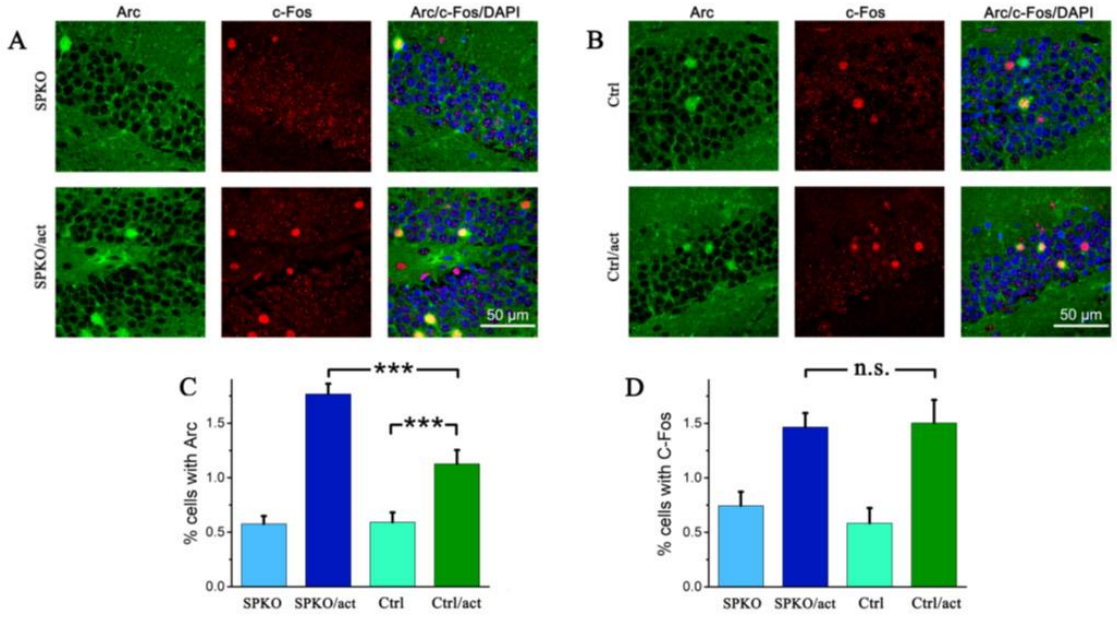

Figure 3. A: Examples of Arc-positive (left, green) and cFos-positive (middle, red) cells and the combined images on background of DAPI-stained cell nucleus (right, blue, merged) in dentate gyrus of the dorsal hippocampus, in control, (top), and active mice (act. bottom). (SPKO n=5, SPKO/act n=7) **B**: same, for wt/heterozygous (control) mice (WT n=1, Het n=2, WT/act n=1, Het/act n=2). Bar 10 µm (size of image 135x135 µm). **C&D** density of arc (C) and c-Fos (D) in the four groups (SPKO n=5; SPKO/act n=7; WT/Hetero n=3, Het n=2; WT/Hetero/act n=3,). Data presented as mean±SEM, ***p<0.01 unpaired Student t-test.

DISCUSSION

The present study addressed a discrepancy among published results on the role of SP in synaptic plasticity. Earlier studies were able to localize SP to the spine apparatus, an enigmatic structure found at a strategic location in the neck of mature dendritic spines. In addition, SP was recently found to be expressed in the soma of activated granule cell in mouse dentate gyrus (Paul et al. 2019), as well as in the cisternal organelle, an integral component of the axon initial segment (Segal, 2018). Subsequent studies using both SP-knockout (SPKO) mice and cells transfected with a fluorescently tagged SP proposed that SP, which is linked to endoplasmic reticulum (ER) is involved in release of calcium from stores, to enable activation of

[Ca^{2+}]i-induced neuronal plasticity (Korkotian and Segal, 2011). This proposition was backed by several studies demonstrating that SPKO animals exhibit impaired cognitive functions in behavioral tests and reduced ability for synaptic plasticity studied in hippocampal slices, in form of long-term potentiation (LTP) (Deller et al, 2003). However, this proposition was confounded by studies showing that LTP and hippocampal dependent learning and memory test in the adult SPKO mouse is actually not different from wt mice, suggesting that older animals are less prone to the SP knockout compared to young brains (Aloni et al. 2019, Jedlicka and Deller, 2017). This observation is puzzling, since older mice are shown to have a higher density of mature spines, spine apparatus and SP puncta than young ones (Czarnecki et al 2005), meaning that SP is likely to serve a more crucial role in sustaining information in the older spines. Furthermore, if indeed SP is less critical at older age, is it replaced by some other molecular families that may facilitate plastic processes primarily in the older animals? To address this question, we embarked on a total RNAseq analysis of the hippocampus of adult, 6-7-month-old wt and SPKO mice. Of the many gene products that were screened we found several that were outstanding and intuitively linked to neuronal plasticity. Far and foremost were the genes for the immediate early genes arc and cFos (Okuno 2011, Ons et al. 2004, Minatohara et al. 2015). Indeed, the basal level of these two genes were higher in all SPKO mice that were examined. Further analysis revealed that SPKO mice exposed to a simple behavioral test expressed a higher elevation of arc, compared to wt mice, indicating that arc may be more involved in plastic processes in the SPKO compared to wt mice. Overall, our results show higher excitability and activity in the adult SPKO mice that may compensate for the reduction of [Ca^{2+}]i in the post synaptic site needed for synaptic plasticity.

In the present study we found that SPKO mice at 6 months of age are hyper-excitable compared to control. This was found in both spike properties and spontaneous PSC's. In an earlier study Bas Orth et al (2007) showed that SPKO animals are not different in several properties of action potentials. Interestingly, while there were no significant differences, the SPKO animals expressed higher firing rates than wt mice. These experiments were conducted with 3 month old mice, while our experiments showing significant differences were conducted with 6 month old mice. This indicates the enhanced excitability matures between 3 and 6 months of age. At the present time it is apparent that SP regulates excitability in both the dendritic spine and the axon initial segment, which is enriched with calcium stores (Segal 2018).

The enhanced excitability in SPKO mice hippocampus brings up an interesting possibility, namely, that SP actually functions to reduce excitability, via activation of some calcium gated K currents. This is hinted in the significantly reduced AHP in the SPKO cells (Fig 2D). Assuming that native SP reduces excitability, how then is this related to its documented function in synaptic plasticity? One possible interpretation is that both LTP and LTD require a rise of [Ca2+]i, even though they produce opposite synaptic action (Mahajan and Nadkarni, 2019). Thus, the rise of [Ca2+]i following stimulation may be employed by postsynaptic mechanisms to control the direction and magnitude of synaptic efficacy.

These issues have important implications with respect to studies which use transgenic animals; when a single molecule is knocked out, can one be sure that this procedure does not affect regulation/expression of

other genes? Can this diverse gene expression affect neuron/network /regulation of functions in the affected animal? Since the early days of gene knockdown, there were many examples of genomic accommodations to a gene knockout, indicating that single gene mutation can lead to a diversity of effects on gene expression. Thus, such studies, relating a single gene to cognitive functions should be interpreted with caution.

Consequently, the electrophysiological changes we observed might result from other homeostatic mechanisms that SPKO cell employ to balance the enhanced excitability and sensitivity of the arc gene. Further investigations are needed to analyze the types of molecular regulators of synaptic and potential properties in these neurons.

REFERENCES:

Aloni, E., E. Oni-Biton, M. Tsoory, D. H. Moallem & M. Segal (2019) Synaptopodin Deficiency Ameliorates Symptoms in the 3xTg Mouse Model of Alzheimer's Disease. *J Neurosci,* 39**,** 3983-3992.

Bas Orth C, Schultz C, Müller CM, Frotscher M, Deller T. Loss of the cisternal organelle in the axon initial segment of cortical neurons in synaptopodin-deficient mice. J Comp Neurol. 2007 Oct 10;504(5):441-9. doi: 10.1002/cne.21445

Czarnecki, K., C. A. Haas, C. Bas Orth, T. Deller & M. Frotscher (2005) Postnatal development of synaptopodin expression in the rodent hippocampus. *J Comp Neurol,* 490**,** 133-44.

Deller, T., C. Bas Orth, D. Del Turco, A. Vlachos, G. J. Burbach, A. Drakew, S. Chabanis, M. Korte, H. Schwegler, C. A. Haas & M. Frotscher (2007) A role for synaptopodin and the spine apparatus in hippocampal synaptic plasticity. *Ann Anat,* 189**,** 5-16.

Deller, T., M. Korte, S. Chabanis, A. Drakew, H. Schwegler, G. G. Stefani, A. Zuniga, K. Schwarz, T. Bonhoeffer, R. Zeller, M. Frotscher & P. Mundel (2003) Synaptopodin-deficient mice lack a spine apparatus and show deficits in synaptic plasticity. *Proc Natl Acad Sci U S A,* 100**,** 10494-9.

Deller, T., T. Merten, S. U. Roth, P. Mundel & M. Frotscher (2000) Actin-associated protein synaptopodin in the rat hippocampal formation: localization in the spine neck and close association with the spine apparatus of principal neurons. *J Comp Neurol,* 418**,** 164-81.

Harris, K. M. (1999) Calcium from internal stores modifies dendritic spine shape. *Proc Natl Acad Sci U S A,* 96**,** 12213-5.

Jedlicka, P. & T. Deller (2017) Understanding the role of synaptopodin and the spine apparatus in Hebbian synaptic plasticity - New perspectives and the need for computational modeling. *Neurobiol Learn Mem,* 138**,** 21-30.

Korkotian, E. & M. Segal (2011) Synaptopodin regulates release of calcium from stores in dendritic spines of cultured hippocampal neurons. *J Physiol,* 589**,** 5987-95.

Mahajan G., Nadkarni S. Intracellular calcium stores mediate metaplasticity at hippocampal dendritic spines. J Physiol. 2019 Jul;597(13):3473-3502. doi: 10.1113/JP277726. Epub 2019 Jun 2.

Minatohara, K., M. Akiyoshi & H. Okuno (2015) Role of Immediate-Early Genes in Synaptic Plasticity and Neuronal Ensembles Underlying the Memory Trace. *Front Mol Neurosci,* 8**,** 78.

Mundel, P., H. W. Heid, T. M. Mundel, M. Krüger, J. Reiser & W. Kriz (1997) Synaptopodin: an actin-associated protein in telencephalic dendrites and renal podocytes. *J Cell Biol,* 139**,** 193-204.

Okubo-Suzuki, R., D. Okada, M. Sekiguchi & K. Inokuchi (2008) Synaptopodin maintains the neural activity-dependent enlargement of dendritic spines in hippocampal neurons. *Mol Cell Neurosci,* 38**,** 266-76.

Okuno, H. (2011) Regulation and function of immediate-early genes in the brain: beyond neuronal activity markers. *Neurosci Res,* 69**,** 175-86.

Ons, S., O. Martí & A. Armario (2004) Stress-induced activation of the immediate early gene Arc (activity-regulated cytoskeleton-associated protein) is restricted to telencephalic areas in the rat brain: relationship to c-fos mRNA. *J Neurochem,* 89**,** 1111-8.

Paul, M. H., M. Choi, J. Schlaudraff, T. Deller & D. Del Turco (2019) Granule Cell Ensembles in Mouse Dentate Gyrus Rapidly Upregulate the Plasticity-Related Protein Synaptopodin after Exploration Behavior. *Cereb Cortex*.

Segal M. Calcium stores regulate excitability in cultured rat hippocampal neurons. J Neurophysiol. 2018 Nov 1;120(5):2694-2705. doi: 10.1152/jn.00447.2018.

Segal, M., A. Vlachos & E. Korkotian (2010) The spine apparatus, synaptopodin, and dendritic spine plasticity. *Neuroscientist,* 16**,** 125-31.

Verbich D., Becker D., Vlachos A., Mundel P., Deller T. and R. Anne McKinney Rewiring (2016) Neuronal microcircuits of the brain via spine head protrusions-a role for synaptopodin and intracellular calcium stores. Acta Neuropathologica Communications, 4, 38.

Vlachos, A., E. Korkotian, E. Schonfeld, E. Copanaki, T. Deller & M. Segal (2009) Synaptopodin regulates plasticity of dendritic spines in hippocampal neurons. *J Neurosci,* 29**,** 1017-33.

Zhang, X. L., B. Pöschel, C. Faul, C. Upreti, P. K. Stanton & P. Mundel (2013) Essential role for synaptopodin in dendritic spine plasticity of the developing hippocampus. *J Neurosci,* 33**,** 12510-8.