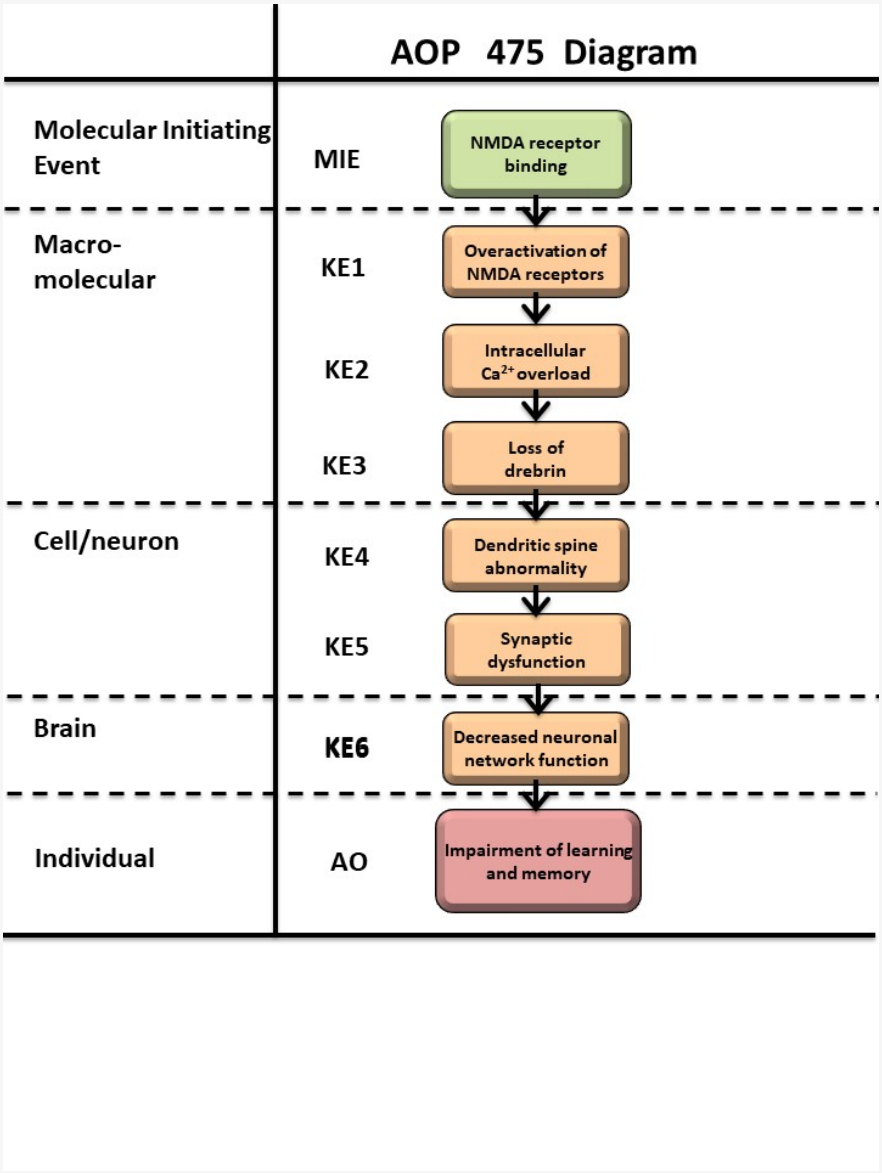
## AOP ID and Title:

AOP 475: Binding of chemicals to ionotropic glutamate receptors leads to impairment of learning and memory via loss of drebrin from dendritic spines of neurons
**Short Title: IGR binding leads to impairment of learning and memory (via loss of drebrin)**

## Graphical Representation

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## Status

| Author status | OECD status | OECD project | SAAOP status |
|---|---|---|---|
| Under development: Not open for comment. Do not cite | Under Development | 1.107 | Included in OECD Work Plan |

## Coaches

Rex FitzGerald

## Abstract

Neurotoxicity risk assessment is crucial for regulatory agencies, as current methods rely on time-consuming and costly animal testing. With thousands of chemicals lacking neurotoxicity data, there is an urgent need for in vitro methods to rapidly evaluate potential risks. Chemicals that impair learning and memory are linked to neurodegenerative diseases such as Parkinson's and Alzheimer's, underscoring the importance of effective risk assessment. The proposed AOP highlights a cascade where the loss of drebrin from dendritic spines induces spine morphological abnormalities, leading to synaptic dysfunction. Notably, synaptic dysfunction alone, even in the absence of neuronal death, can result in learning and memory impairments. This provides a novel framework for evaluating neurotoxicity and developmental neurotoxicity.

Dendritic spines are specialized structures that serve as the primary sites of excitatory synaptic transmission, primarily mediated by glutamate receptors. Spine formation and functional maturation are governed by drebrin expression. Drebrin, an actin-binding protein discovered and named by Shirao's team, has two isoforms: drebrin E (DE) and drebrin A (DA). DE, a non-neuronal protein involved in cell motility and protrusion formation, is predominantly expressed during early brain development. It is gradually replaced by DA, a neuron-specific protein that stabilizes actin filaments during synaptogenesis and synaptic maturation. The protein levels and subcellular localization of drebrin reflect its distinct roles in neuronal development and synaptic function. Loss of drebrin triggers spine abnormalities and synaptic dysfunction, ultimately impairing learning and memory as the adverse outcome (AO). This AOP builds on the molecular initiating event (MIE) of AOP 48—“Binding of agonists to ionotropic glutamate receptors”—but uniquely highlights drebrin loss as a critical KE.

Empirical evidence supports this AOP. Studies show that glutamate induces drebrin loss and dendritic spine morphological changes. Compounds that directly bind to NMDA receptors, such as glutamate and NMDA, and compounds that indirectly enhance NMDA receptor activity, have been shown to induce drebrin loss, linking such exposure to synaptic dysfunction and learning impairments. The detection of drebrin gene expression levels, subcellular localization, and protein levels provides valuable insights into brain development and higher-order functions across species. To develop effective in vitro testing methods, it is essential to have biomarkers that are simple, reproducible, and allow for quantitative data analysis. We determined that drebrin is highly suitable as a biomarker for these purposes.

The proposed AOP promotes alternative testing methods aligned with the 3Rs (Replacement, Reduction, Refinement), using cryopreserved hippocampal neurons to reduce animal use. Quantification of drebrin via immunocytochemistry and ELISA enables reproducible and scalable chemical screening. This AOP provides a foundation for in vitro prediction models, advancing chemical safety evaluation for humans and the environment. By integrating dynamic drebrin expression patterns and functional properties, this AOP framework offers a robust tool for regulatory decision-making and assessing neurotoxicity risks.

## AOP Development Strategy

### Context

Drebrin, identified by our group in 1985, has been a central focus of our research for many years. This actin-binding protein is known to exist in two isoforms: drebrin E and drebrin A. Drebrin E is expressed in both neuronal and certain non-neuronal cells, and it plays a crucial role during fetal and early postnatal stages of brain development. In neurons, drebrin E is involved in processes essential for cell motility, neurite outgrowth, and the extension of axons and dendrites, which together contribute to the establishment of neural networks. In contrast, drebrin A is a neuron-specific isoform whose expression is initiated during the synaptic formation stage. Drebrin A is indispensable for the formation and maintenance of dendritic spines, which serve as the primary sites for excitatory synaptic transmission, largely mediated by glutamate receptors. During brain development, drebrin E, which predominates in the early stages, is gradually replaced by drebrin A during synaptogenesis, reflecting their distinct roles in neuronal development, excitatory synapse formation and synaptic plasticity.

Based on the dynamic expression patterns and functional properties of drebrin, we have proposed an Adverse Outcome Pathway (AOP) framework for assessing developmental neurotoxicity and neurotoxicity. Monitoring drebrin gene expression levels, subcellular localization in neurons, and protein levels provides valuable insights into brain development and higher-order brain functions across various species.

In 2017, we summarized our research findings in a monograph titled*Drebrin: From Structure and Function to Physiological and Pathological Roles*, published as part of Springer’s*Advances in Experimental Medicine and Biology* series.

Between 2020 and 2023, we initiated the development of an AOP for neurotoxicity and developmental neurotoxicity caused by glutamate receptor-binding agonists, supported by a three-year research grant from the Japan Chemical Industry Association (JCIA) Long-range Research Initiative (LRI). This project, entitled "Proposal of a new AOP for the neurotoxicity and developmental neurotoxicity assessment of glutamate receptor binding agonists that cause learning and memory impairment," identified a novel adverse outcome (AO): learning and memory impairment. This AO is characterized by key events, including the loss of drebrin from dendritic spines, leading to thin and elongated spine morphology and subsequent synaptic dysfunction. Drebrin, as a key regulator of dendritic spine morphology, plays an essential role in the structural plasticity associated with learning and memory. Furthermore, the subcellular localization of drebrin is dynamically influenced by glutamate receptor activity.

To advance these studies, we optimized Banker’s method for low-density neuronal cultures and developed an immunocytochemical evaluation system for drebrin clusters in hippocampal neurons using a 96-well plate format and frozen embryonic hippocampal neurons from rats. In addition, we designed a high-content imaging algorithm for the quantitative analysis of neuronal parameters, including neuron count, dendrite length, and drebrin clustering, using confocal image cytometry. Notably, our brightness distribution analysis of drebrin clusters demonstrated exceptional sensitivity, allowing for precise quantification of structural changes in neurons. These methodological advancements have enabled us to establish standardized protocols (SOPs) for both neuronal culture techniques and analytical procedures.

We plan to extend this research by developing an experimental system utilizing neurons derived from human-induced pluripotent stem cells (iPSCs), thereby enhancing the relevance and applicability of our findings to human brain development and function.

### Strategy

Drebrin is an essential protein for the formation of dendritic spines, which are structural compartment modules of the protein network of learning and memory. Evidence from in vivo experiments and clinical observations indicates a strong association between the loss of drebrin and memory impairment, as the molecular mechanisms of learning do not function in spines lacking drebrin. By staining cultured

neurons with drebrin using immunocytochemistry, the number of drebrin clusters with a certain threshold intensity can be quantified, and thus the loss of drebrin from dendritic spines can be detected. When NMDA-type glutamate receptors are activated, drebrin translocates from dendritic spines to dendritic trunks. This process is accompanied by a change in spine morphology from a mushroom shape to a thin and pointed shape. We proposed the disappearance of drebrin (KE2028) as a new KE following a molecular initiation event (MIE; Event ID 875) in which a compound binds to the glutamate NMDA-type receptor, followed by NMDA receptor overactivation (KE388) and intracellular calcium overload (KE389). Loss of drebrin leads to abnormal dendritic spine morphology (KE2242) and associated synaptic dysfunction (KE1944).

## Summary of the AOP

### Events

### Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

| Sequence | Type | Event ID | Title | Short name |
|---|---|---|---|---|
| | MIE | 875 | Binding of agonist, Ionotropic glutamate receptors | Binding of agonist, Ionotropic glutamate receptors |
| | KE | 388 | Overactivation, NMDARs | Overactivation, NMDARs |
| | KE | 389 | Increased, Intracellular Calcium overload | Increased, Intracellular Calcium overload |
| | KE | 2078 | Loss of drebrin | Loss of drebrin |
| | KE | 2242 | Abnormality, dendritic spine morphology | Dendritic spine abnormality |
| | KE | 1944 | Synaptic dysfunction | Dysfunctional synapses |
| | KE | 386 | Decrease of neuronal network function | Neuronal network function, Decreased |
| | AO | 341 | Impairment, Learning and memory | Impairment, Learning and memory |

### Key Event Relationships

| Upstream Event | Relationship Type | Downstream Event | Evidence | Quantitative Understanding |
|---|---|---|---|---|
| Binding of agonist, Ionotropic glutamate receptors | adjacent | Overactivation, NMDARs | High | Moderate |
| Overactivation, NMDARs | adjacent | Increased, Intracellular Calcium overload | High | High |
| Increased, Intracellular Calcium overload | adjacent | Loss of drebrin | High | Moderate |
| Loss of drebrin | adjacent | Abnormality, dendritic spine morphology | High | High |
| Abnormality, dendritic spine morphology | adjacent | Synaptic dysfunction | High | High |
| Synaptic dysfunction | adjacent | Decrease of neuronal network function | High | Moderate |
| Decrease of neuronal network function | adjacent | Impairment, Learning and memory | High | Moderate |
| Loss of drebrin | non-adjacent | Impairment, Learning and memory | High | High |
| Binding of agonist, Ionotropic glutamate receptors | non-adjacent | Loss of drebrin | High | High |

### Stressors

| Name | Evidence |
|---|---|
| Sodium L-glutamate | |
| amyloid beta | |
| Iotenone | |
| N-Methyl-D-aspartic acid | |

## Overall Assessment of the AOP

The overall assessment of AOP475 emphasizes its relevance in evaluating neurodevelopmental toxicity caused by chemical exposures. The pathway links disruption of dendritic spine morphology to impaired synaptic plasticity and cognitive functions, such as learning and memory. Its robust mechanistic framework integrates molecular, cellular, and behavioral endpoints. AOP475 serves as a critical tool for regulatory risk assessment, particularly in identifying early indicators of synaptic dysfunction.

### Domain of Applicability

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| Fetal | High |
| Perinatal | High |
| During brain development | High |
| Adult | High |
| Old Age | High |
| Juvenile | High |

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| human | Homo sapiens | Moderate | NCBI |
| mouse | Mus musculus | High | NCBI |
| rat | Rattus norvegicus | High | NCBI |
| Caenorhabditis elegans | Caenorhabditis elegans | Moderate | NCBI |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Male | High |
| Female | High |

**Taxa:**
Drebrin has been primarily studied in mammals, with its expression confirmed in vertebrates such as humans, mice, rats and C-elegans.

**Sex:**
No sex differences in the expression or function of drebrin have been reported. Therefore, its applicability is not influenced by sex.

**Life Stage:**
Drebrin has two main isoforms: drebrin E, which is predominantly expressed during the fetal and juvenile stages, and drebrin A, which is specific to the mature stage. This suggests that drebrin's role varies depending on the life stage.

## Essentiality of the Key Events

AOP475 describes a series of biological changes leading from the molecular initiating event (MIE), where glutamate receptor agonists bind to their receptors, to the adverse outcome (AO) of learning and memory impairment. Each Key Event (KE) plays an essential role in the progression of this pathway, and the causal relationships between these events are supported by experimental evidence and published literature. The following summarizes the Essentiality of Key Events in AOP475:

**Molecular Initiating Event (MIE): Binding of agonist, Ionotropic glutamate receptors : Event ID MIE 875**

This is the primary trigger for all subsequent Key Events, initiating changes in calcium homeostasis and cellular signaling. The involvement of NMDAR in neurotoxicity has been extensively documented in the literature.

**KE1: Overactivation, NMDAR (KE 388)**

NMDAR overactivation is a well-characterized phenomenon in excitotoxicity and has been supported by both experimental studies and reviews discussing its role in calcium dysregulation and neurodegeneration.

**KE2: Increase, Intracellular Calcium overload (KE389)**

Overactivation of NMDAR causes a persistent increase in intracellular calcium levels, disrupting calcium homeostasis. This dysregulation impacts cytoskeletal dynamics, including actin remodeling, and is critical for the subsequent loss of Drebrin from dendritic spines. Evidence for calcium's role in synaptic and structural stability has been widely reported in the literature.

**KE3: Loss of Drebrin from Dendritic Spines ( KE 2078 : a new key event )**

Drebrin is essential for maintaining spine morphology and synaptic plasticity. Its loss from dendritic spines has been consistently observed in experimental models, and its significance is well-supported by publications emphasizing its role in spine stability and cognitive function.

**KE4:Abnormalities Dendritic Spine Morphological (KE2242: a new key event)**

Dendritic spines become thin and elongated, losing their structural stability. Morphological changes in spines impair excitatory synaptic transmission and plasticity. These abnormalities are well-documented in both experimental findings and studies on neurodegenerative diseases.

**KE5: Synaptic Dysfunction (KE1944: a new key event )**

Becasue denddritc spine is a structure that receive glutamate signals via glutamate receptoes. Synaptic transmission efficiency declines, reducing the ability of neurons to communicate effectively. Synaptic dysfunction, including reduced synaptic strength and plasticity, has been confirmed through electrophysiological studies and corroborated by literature focusing on the role of spines in neural networks.

**KE6: Decrease of Neuronal Network Function**

Network-level impairments are supported by experimental models and computational studies, as well as publications addressing the effects of synaptic disruptions on overall brain function.

**Adverse Outcome (AO): Learning and Memory Impairment (AO 341)**

The adverse outcome is the culmination of upstream events, supported by behavioral studies and widely recognized in reviews discussing neurotoxicity and cognitive decline.

### Weight of Evidence Summary

**Biological plausibility:** In AOP 475, chemical stimulation of glutamate receptors, particularly NMDA receptors, results in excessive intracellular calcium influx. This calcium overload leads to a loss of drebrin from dendritic spines through several possible mechanisms, such as translocation via acto-myosin interaction from dendritic spines to dendritic shafts, degradation by calpain, the ubiquitin-proteasome system (UPS) and caspases, calcineurin-dependent dephosphorylation leading to drebrin destabilization, and supression of drebrin synathesis through inhibition of mRNA translation. Under normal physiological conditions, drebrin binds to actin within dendritic spines, stabilizing spine morphology. Temporary drebrin reduction occurs even during physiological NMDA receptor activation and calcium influx; however, drebrin returns to dendritic spines during long-term potentiation (LTP), stabilizing newly inserted receptors and slightly enlarging spine morphology. In contrast, prolonged drebrin loss during long-term depression (LTD) changed spine morphokogy and enhances endocytosis, reducing PSD95 and glutamate receptors. Pathological conditions exacerbate drebrin loss, which is implicated in memory impairment associated with Alzheimer's disease (AD).

**Empirical Support:** Drebrin reduction begins during mild cognitive impairment (MCI), an early stage of AD. Experimentally induced drebrin reduction via genetic manipulation or radiation exposure results in learning and memory deficits, which can be reversed upon restoration of drebrin levels. Furthermore, animal models of Alzheimer's disease also exhibit decreased drebrin levels.

**Quantitative understanding:**

Existing experimental studies have quantitatively demonstrated that prolonged or excessive activation of NMDA receptors leads to measurable increases in intracellular calcium levels, which correlate with significant reductions in drebrin levels in dendritic spines. There is a quantitative relationship between the dose of glutamate applied to neuron cultures and the reduction of the number of drebrin clusters. Duration of the glutamate treatment showed shifts of the dose-response curve to the left.Quantitative data from immunocytochemical assays using cultured neurons have also shown dose-dependent decreases in drebrin cluster density following exposure to NMDA receptor agonists or neurotoxicants. Additionally, quantitative correlations between the extent of drebrin loss and the severity of cognitive impairment have been reported in animal models and clinical studies, underscoring the potential to derive quantitative thresholds or benchmarks useful in chemical risk assessment.

### Quantitative Consideration

Drebrin loss has dose-response relationships to concentration of glutamate, depending on the period of the trearment.

Cognitive impairment has dode-respose to drebrin level in the brain.

## Considerations for Potential Applications of the AOP (optional)

AOP475 provides a framework useful in risk assessments of regulatory toxicology.

KE 2078, drebrin loss, can be quantitatively measured using immunocytochemistry. Thus, targeted assays could be developed to evaluate chemicals' potential to disrupt synaptic function and cognitive performance. Quantitative measurement of the number of drebrin clusters, assessed by immunohistochemistry using neuronal cultures derived from frozen embryonic neurons, could be integrated into an IATA framework to provide a comprehensive neurotoxicity assessment, reducing reliance on animal testing.

## References


1. Shirao T., Sekino Y. (Eds.) (2017) Drebrin. (Advances in Experimental Medicine and Biology, vol 1006) Tokyo, Springer
2. Adverse Outcome Pathway on binding of agonists to ionotropic glutamate receptors in adult brain leading to excitotoxicity that mediates neuronal cell death, contributing to learning and memory impairment, Sachana M, et al. OECD Series on Adverse Outcome Pathways (2016) No. 6, OECD Publishing, Paris, doi: 10.1787/5jlr8vqgm630-en.
3. Synapse pathology in Alzheimer’s disease, Griffiths J, Grant GN. Seminars in Cell and Developmental Biology (2022) doi: 10.1016/j.semcdb.2022.05.028. (review)
4. Dopamine Restores Limbic Memory Loss, Dendritic Spine Structure, and NMDAR-Dependent LTD in the Nucleus Accumbens of Alcohol-Withdrawn Rats Cannizzaro C, et al. J Neurosci. (2019) Jan 30;39(5):929-943. doi: 10.1523/JNEUROSCI.1377-18.2018.
5. Actin in dendritic spines: connecting dynamics to function, Hotulainen P, Hoogenraad C. J Cell Biol (2010) 17;189(4):619-29. doi: 10.1083/jcb.201003008. (review)
6. Role of actin cytoskeleton in dendritic spine morphogenesis. Sekino Y, et al. Neurochem Int. (2007) 51(2-4):92-104. doi: 10.1016/j.neuint.2007.04.029. (review)
7. Drebrin, a dendritic spine protein, is manifold decreased in brains of patients with Alzheimer’s disease and Down syndrome Shim KS, Lubec G. Neurosci Lett. 2002 May 24;324(3):209-12. doi: 10.1016/s0304-3940(02)00210-0.
8. Differential expression of synaptic proteins in the frontal and temporal cortex of elderly subjects with mild cognitive impairment, Counts SE, et al. J Neuropathol Exp Neurol. 2006 Jun;65(6):592-601. doi: 10.1097/00005072-200606000-00007.
9. High-content imaging analysis for detecting the loss of drebrin clusters along dendrites in cultured hippocampal neurons Hanamura K, et al. J Pharmacol Toxicol Methods. (2019) 99:106607. doi: 10.1016/j.vascn.2019.106607.
10. Assessment of NMDA receptor inhibition of phencyclidine analogues using a high-throughput drebrin immunocytochemical assay Mitsuoka T, et al. J Pharmacol Toxicol Methods. (2019) 99:106583. doi: 10.1016/j.vascn.2019
11. Genetic disruption of the alternative splicing of drebrin gene impairs context-dependent fear learning in adulthood, Kojima N, et al. Neuroscience. (2010) 165(1):138-50. Doi: 10.1016/j.neuroscience.2009.10.016.
12. Drebrin A regulates hippocampal LTP and hippocampus-dependent fear learning in adult mice, Kojima N, et al. Neuroscience. (2016) Jun 2;324:218-26. doi: 10.1016/j.neuroscience.2016.03.015.
13. Effective expression of Drebrin in hippocampus improves cognitive function and alleviates lesions of Alzheimer’s disease in APP (swe)/PS1 (ΔE9) mice, Liu Y, et al. CNS Neurosci Ther. (2017) Jul;23(7):590-604. doi: 10.1111/cns.12706.


## Appendix 1

### List of MIEs in this AOP

## Event: 875: Binding of agonist, Ionotropic glutamate receptors

### Short Name: Binding of agonist, Ionotropic glutamate receptors

### Event Component

| Process | Object | Action |
|---|---|---|
| ionotropic glutamate receptor activity | ionotropic glutamate receptor complex | increased |

### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:48 - Binding of agonists to ionotropic glutamate receptors in adult brain causes excitotoxicity that mediates neuronal cell death, contributing to learning and memory impairment. | MolecularInitiatingEvent |
| Aop:475 - Binding of chemicals to ionotropic glutamate receptors leads to impairment of learning and memory via loss of drebrin from dendritic spines of neurons | MolecularInitiatingEvent |

### Stressors

| Name |
|---|
| Domoic acid |

### Biological Context

**Level of Biological Organization**

Molecular

### Cell term

**Cell term**

neuron

### Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| Drosophila melanogaster | Drosophila melanogaster | High | NCBI |
| Rattus norvegicus | Rattus norvegicus | High | NCBI |
| Primates sp. BOLD:AAA0001 | Primates sp. BOLD:AAA0001 | High | NCBI |
| human | Homo sapiens | High | NCBI |
| mice | Mus sp. | High | NCBI |

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The major determinants for ligand e.g. for both co-agonist glycine binding and L-glutamate binding are well conserved between species from lower organism to mammals (reviewed in Xia and Chiang, 2009). PCR analysis, cloning and subsequent sequencing of the seal lion NMDA receptors showed 80% homology to those from rats, but more than 95% homologus to those from dogs (Gill et al., 2010).

### Key Event Description

The MIE of this AOP can be triggered by direct binding of an agonist to NMDARs or indirectly through initial activation of KA/AMPARs. Indeed, binding of agonist to KA/AMPARs results in ion influx (Na+ and a small efflux of K+) and glutamate release from excitatory synaptic vesicles causing depolarization of the postsynaptic neuron (Dingledine et al. 1999). Upon this depolarization the Mg2+ block is removed from the pore of NMDARs, allowing sodium, potassium, and importantly, calcium ions to enter into a cell. At positive potentials NMDARs then show maximal permeability (i.e., large outward currents can be observed under these circumstances). Due to the time needed for the Mg2+ removal, NMDARs activate more slowly, having a peak conductance long after the KA/AMPAR peak conductance takes place. It is important to note that NMDARs conduct currents only when Mg2+ block is relieved, glutamate is bound, and the postsynaptic neuron is depolarized. For this reason the NMDA receptors act as “coincidence detectors” and play a fundamental role in the establishment of Hebbian synaptic plasticity which is considered the physiological correlate of associative learning (Daoudal and Debanne, 2003; Glanzman, 2005). Post-synaptic membrane depolarization happens almost always through activation of KA/AMPARs (Luscher and Malenka, 2012). Therefore, a MIE of this AOP is defined as binding of an agonist to these three types of ionotropic receptors (KA/AMPA and NMDA) that can result in a prolonged overactivation of NMDARs through (a) direct binding of an agonist or (b) indirect, mediated through initial KA/AMPARs activation. The excitotoxic neuronal cell death, triggered by sustained NMDARs overactivation in the hippocampus and/or cortex leads to the impaired learning and memory, defined as the adverse outcome (AO) of this AOP.

**Biological state:** L-glutamate (Glu) is a neurotransmitter with important role in the regulation of brain development and maturation processes. Two major classes of Glu receptors, ionotropic and metabotropic, have been identified. Due to its physiological and pharmacological properties, Glu activates three classes of ionotropic receptors named: α-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid (AMPA receptors), 2-carboxy-3-carboxymethyl-4-isopropenylpyrrolidine (kainate receptors) and N-methyl-D-aspartate (NMDA receptors, NMDARs), which transduce the postsynaptic signal. Ionotropic glutamate receptors are integral membrane proteins formed by four large subunits that compose a central ion channel pore. In case of NMDA receptors, two NR1 subunits are combined with either two NR2 (NR2A, NR2B, NR2C, NR2D) subunits and less commonly are assembled together with a combination of NR2 and NR3 (A, B) subunits (reviewed in Traynelis et al., 2010). To be activated NMDA receptors require simultaneous binding of both glutamate to NR2 subunits and of glycine to either NR1 or NR3 subunits that provide the specific binding sites named extracellular ligand-binding domains (LBDs). Apart from LBDs, NMDA receptor subunits contain three more domains that are considered semiautonomous: 1) the extracellular amino-terminal domain that plays important role in assembly and trafficking of these receptors; 2) the transmembrane domain that is linked with LBD and contributes to the formation of the core of the ion channel and 3) the intracellular carboxyl-terminal domain that influences membrane targeting, stabilization, degradation and post-translation modifications.

**Biological compartments:** The genes of the NMDAR subunits are expressed in various tissues and are not only restricted to the nervous system. The level of expression of these receptors in neuronal and non-neuronal cells depends on: transcription, chromatin remodelling, mRNA levels, translation, stabilization of the protein, receptor assembly and trafficking, energy metabolism and numerous environmental stimuli (reviewed in Traynelis et al., 2010). In hippocampus region of the brain, NR2A and NR2B are the most abundant NR2 family subunits. NR2A-containing NMDARs are mostly expressed synaptically, while NR2B-containing NMDARs are found both synaptically and extrasynaptically (Tovar and Westbrook, 1999).

**General role in biology:** NMDA receptors, when compared to the other Glu receptors, are characterized by higher affinity for Glu, slower activation and desensitisation kinetics, higher permeability for calcium (Ca2+) and susceptibility to potential-dependent blockage by magnesium ions (Mg2+). NMDA receptors are involved in fast excitatory synaptic transmission and neuronal plasticity in the central nervous system (CNS). Functions of NMDA receptors:

1. They are involved in cell signalling events converting environmental stimuli to genetic changes by regulating gene transcription and epigenetic modifications in neuronal cells (Cohen and Greenberg, 2008).

2. In NMDA receptors, the ion channel is blocked by extracellular Mg2+ and Zn2+ ions, allowing the flow of Na+ and Ca2+ ions into the cell and K+ out of the cell which is voltage-dependent. Ca2+ flux through the NMDA receptor is considered to play a critical role in pre- and post-synaptic plasticity, a cellular mechanism important for learning and memory (Barria and Malinow, 2002).

3. The NMDA receptors have been shown to play an essential role in the strengthening of synapses and neuronal differentiation, through long-term potentiation (LTP), and the weakening of synapses, through long-term depression (LTD). All these processes are implicated in the memory and learning function (Barria and Malinow, 2002).

### How it is Measured or Detected

*Methods that have been previously reviewed and approved by a recognized authority should be included in the Overview section above. All other methods, including those well established in the published literature, should be described here. Consider the following criteria when describing each method: 1. Is the assay fit for purpose? 2. Is the assay directly or indirectly (i.e. a surrogate) related to a key event relevant to the final adverse effect in question? 3. Is the assay repeatable? 4. Is the assay reproducible?*

1. Ex vivo: The most common assay used is the NMDA receptor (MK801 site) radioligand competition binding assay (Reynolds and Palmer, 1991; Subramaniam and McGonigle, 1991; http://pdsp.med.unc.edu/UNC-CH%20Protocol%20Book.pdf; http://www.currentprotocols.com/WileyCDA/CPUnit/refId-ph0120.html). This assay is based on the use of the most potent and specific antagonist of this receptor, MK801 that is used to detect and differentiate agonists and antagonists (competitive and non-competitive) that bind to this specific site of the receptor. Also radioligand competition binding assay can be performed using D, L-(E)-2-amino-4-[3H]-propyl-5-phosphono-3-pentenoic acid ([3H]-CGP 39653), a high affinity selective antagonist at the glutamate site of NMDA receptor, which is a quantitative autoradiography technique (Mugnaini et al., 1996). D-AP5, a selective N-methyl-D-aspartate (NMDA) receptor antagonist that competitively inhibits the glutamate binding site of NMDA receptors, can be studied by evoked electrical activity measurements. AP5 has been widely used to study the activity of NMDA receptors particularly with regard to researching synaptic plasticity, learning, and memory (Evans et al.,1982; Morris, 1989). The saturation binding of radioligands are used to measure the affinity (Kd) and density (Bmax) of kainate and AMPA receptors in striatum, cortex and hippocampus (Kürschner et al., 1998).

2. In silico: The prediction of NMDA receptor targeting is achievable by combining database mining, molecular docking, structure-based pharmacophore searching, and chemical similarity searching methods together (Neville and Lytton, 1999; Mazumder Borah, 2014)

### References

(for Abstract and MIE)

Barenberg P, Strahlendorf H, Strahlendorf., Hypoxia induces an excitotoxic-type of dark cell degeneration in cerebellar Purkinje neurons. J. Neurosci Res. 2001, 40(3): 245-54.

Barria A, Malinow R. (2002) Subunit-specific NMDA receptor trafficking to synapses. Neuron 35: 345-353. Cohen S, Greenberg ME. (2008) Communication between the synapse and the nucleus in neuronal development, plasticity, and disease. Ann Rev Cell Dev Biol 24: 183-209.

Berman F.W. and T. F. Murray, “Domoic acid neurotoxicity in cultured cerebellar granule neurons is mediated predominantly by NMDA receptors that are activated as a consequence of excitatory amino acid release,” Journal of Neurochemistry, 1997, 69: 693–703.

Berman W.F., K. T. LePage, and T. F. Murray, Domoic acid neurotoxicity in cultured cerebellar granule neurons is controlled preferentially by the NMDA receptor Ca2+ influx pathway,” Brain Research, 2002, 924: 20–29.

Cohen S, Greenberg ME. (2008) Communication between the synapse and the nucleus in neuronal development, plasticity, and disease. Ann Rev Cell Dev Biol 24: 183-209.

Daoudal G, Debanne D, Long-term plasticity of intrinsic excitability: learning rules and mechanisms. Learn Mem., 2003, 10(6):456-65.

Dingledine R, Borges K, Bowie D, Traynelis SF., The glutamate receptor ion channels. Pharmacol Rev., 1999, 51: 7–61.

Evans, R.H., Francis, A.A., Jones, A.W., et al., The Effects of a Series of ω-Phosphonic α-Carboxylic Amino Acids on Electrically Evoked and Excitant Amino Acid-Induced Responses in Isolated Spinal Cord Preparations. Br J Pharmac., 1982, 75: 65-75.

Gill S, Goldstein T, Situ D, Zabka TS, Gulland FM, Mueller RW., Cloning and characterization of glutamate receptors in Californian sea lions (Zalophus californianus). Mar Drugs, 2010, 8: 1637-1649.

Glanzman DL., Associative learning: Hebbian flies. Curr Biol., 2005, 7: 15(11):R416-9.

Kürschner VC, Petruzzi RL, Golden GT, Berrettini WH, Ferraro TN., Kainate and AMPA receptor binding in seizure-prone and seizure-resistant inbred mouse strains. Brain Res. 1998, 5: 780-788.

Lantz SR, Mack CM, Wallace K, Key EF, Shafer TJ, Casida JE., Glufosinate binds N-methyl-D-aspartate receptors and increases neuronal network activity in vitro. Neurotoxicology, 2014, 45: 38-47.

Lefebvre KA, Robertson A., Domoic acid and human exposure risks: a review.Toxicon. 2010, 56: 218-30.

Luscher C. and Robert C. Malenka., NMDA Receptor-Dependent Long-Term Potentiation and Long-Term Depression (LTP/LTD). Cold Spring Harb Perspect Biol 2012, 4: a005710.

Matsumura N, Takeuchi C., Hishikawa K, Fujii T, Nakaki T., Glufosinate ammonium induces convulsion through N-methyl-D-aspartate receptors in mice. Neurosci Lett. 2001, 304: 123-5.

Mazumder MK, Borah A. Piroxicam inhibits NMDA receptor-mediated excitotoxicity through allosteric inhibition of the GluN2B subunit: an in silico study elucidating a novel mechanism of action of the drug. Med Hypotheses. 2014, 83(6): 740-6.

Mehta A, Prabhakar M, Kumar P, Deshmukh R, Sharma PL. Excitotoxicity: bridge to various triggers in neurodegenerative disorders. Eur J Pharmacol. 2013, 698: 6-18.

Morris, RJ. Synaptic Plasticity and Learning: Selective Impairment of Learning in Rats and Blockade of Long-Term Potentiation in vivo by the N-Methyl-D-Aspartate Receptor Antagonist AP5. J Neurosci., 1989, 9: 3040-3057.

Mugnaini M, van Amsterdam FT, Ratti E, Trist DG, Bowery NG. Regionally different N-methyl-D-aspartate receptors distinguished by ligand binding and quantitative autoradiography of [3H]-CGP 39653 in rat brain.British Journal of Pharmacology, 1996, 119: 819–828.

Neville KR, Lytton WW. Potentiation of Ca2+ influx through NMDA channels by action potentials: a computer model. Neuroreport., 1999, 10(17): 3711-6.

Pulido OM., Domoic acid toxicologic pathology: a review. Mar Drugs., 2008, 6: 180-219.

Reynolds IJ, Palmer AM. Regional variations in [3H]MK801 binding to rat brain N-methyl-D-aspartate receptors. J Neurochem. 1991, 56(5):1731-40.

Subramaniam S, McGonigle P. Quantitative autoradiographic characterization of the binding of (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5, 10-imine ([3H]MK-801) in rat brain: regional effects of polyamines. J Pharmacol Exp Ther. 1991, 256(2): 811-9.

Schrattenholz A, Soskic V., NMDA receptors are not alone: dynamic regulation of NMDA receptor structure and function by neuregulins and transient cholesterol-rich membrane domains leads to disease-specific nuances of glutamate-signalling.Curr Top Med Chem., 2006, 6(7):663-86.

Tovar KR, Westbrook GL. (1999) The incorporation of NMDA receptors with a distinct subunit composition at nascent hippocampal synapses in vitro. J Neurosci. 19: 4180–4188.

Traynelis S, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, Hansen KB, Yuan H, Myers SJ, Dingledine R. Glutamate receptor ion channels: structure, regulation, and function. Pharmacol Rev., 2010, 62: 405-496.

Watanabe KH, Andersen ME, Basu N, Carvan MJ 3rd, Crofton KM, King KA, Suñol C, Tiffany-Castiglioni E, Schultz IR. Defining and modeling known adverse outcome pathways: Domoic acid and neuronal signaling as a case study. Environ Toxicol Chem., 2011, 30: 9-21.

Xia S, Chiang AS. NMDA Receptors in Drosophila. In: Van Dongen AM, editor. Biology of the NMDA Receptor. Boca Raton (FL): CRC Press; 2009. Chapter 10. Available from: http://www.ncbi.nlm.nih.gov/books/NBK5286/

Retrieved from https://aopkb.org/aopwiki/index.php/?oldid=27027

## List of Key Events in the AOP

### Event: 388: Overactivation, NMDARs

#### Short Name: Overactivation, NMDARs

#### Event Component

| Process | Object | Action |
|---|---|---|
| NMDA glutamate receptor activity | NMDA selective glutamate receptor complex | increased |
| abnormal excitatory postsynaptic current amplitude | dendritic spine membrane | morphological change |

## AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:48 - Binding of agonists to ionotropic glutamate receptors in adult brain causes excitotoxicity that mediates neuronal cell death, contributing to learning and memory impairment. | KeyEvent |
| Aop:281 - Acetylcholinesterase Inhibition Leading to Neurodegeneration | KeyEvent |
| Aop:464 - Calcium overload in dopaminergic neurons of the substantia nigra leading to parkinsonian motor deficits | MolecularInitiatingEvent |
| Aop:475 - Binding of chemicals to ionotropic glutamate receptors leads to impairment of learning and memory via loss of drebrin from dendritic spines of neurons | KeyEvent |
| Aop:471 - Neuron defect induced early behavioral change | KeyEvent |

## Biological Context

**Level of Biological Organization**

Cellular

## Cell term

**Cell term**

neuron

## Organ term

**Organ term**

brain

## Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| human | Homo sapiens | High | NCBI |
| mouse | Mus musculus | High | NCBI |
| rat | Rattus norvegicus | High | NCBI |
| zebrafish | Danio rerio | High | NCBI |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| During brain development, adulthood and aging | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Male | High |

It is important to note that in invertebrates the glutamatergic synaptic transmission has an inhibitory and not an excitatory role like in vertebrates. This type of neurotransmission is mediated by glutamate-gated chloride channels that are members of the ‘cys-loop’ ligand-gated anion channel superfamily found only in invertebrates. The subunits of glutamate-activated chloride channel have been isolated from C. elegans and from Drosophila (Blanke and VanDongen, 2009).

## Key Event Description

**Biological state:** Please see MIE NMDARs, Binding of antagonist

**Biological compartments:** Please see MIE NMDARs, Binding of antagonist

**General role in biology:** Please see MIE NMDARs, Binding of antagonist

The above chapters belong to the AOP entitled: *Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment of learning and memory abilities* since the general characteristic of the NMDA receptor biology is the same for both AOPs.

Additional text, specific for this AOP:

At resting membrane potentials, NMDA receptors are inactive. Depending on the specific impulse train received, the NMDA receptor activation triggers long term potentiation (LTP) or long-term depression (LTD) (Malenka and Bear, 2004; Luscher and Malenka, 2012). LTP (the opposing process to LTD) is the long-lasting increase of synaptic strength. For LTP induction both pre- and postsynaptic neurons need to be active at the same time because the postsynaptic neuron must be depolarized when glutamate is released from the presynaptic bouton to fully relieve the Mg2+ block of NMDARs that prevents ion flows through it. Sustained activation of AMPA or KA

receptors by, for instance, a train of impulses arriving at a pre-synaptic terminal, depolarizes the post-synaptic cell, releasing Mg2+ inhibition and thus allowing NMDA receptor activation. Unlike GluA2-containing AMPA receptors, NMDA receptors are permeable to calcium ions as well as being permeable to other ions. Thus NMDA receptor activation leads to a calcium influx into the post-synaptic cells, a signal that is instrumental in the activation of a number of signalling cascades (*Calcium-dependent processes are describe in Key Event Calcium influx, increased*). Postsynaptic Ca2+ signals of different amplitudes and durations are able to induce either LPT or LTD.

Conversely to LTP, LTD is induced by repeated activation of the presynaptic neuron at low frequencies without postsynaptic activity (Luscher and Malenka, 2012). Therefore, under physiological conditions LTD is one of several processes that serves to selectively weaken specific synapses in order to make constructive use of synaptic strengthening caused by LTP. This is necessary because, if allowed to continue increasing in strength, synapses would ultimately reach a ceiling level of efficiency, which would inhibit the encoding of new information (Purves, 2008).

LTD is an activity-dependent reduction in the efficacy of neuronal synapses lasting hours or longer following a long patterned stimulus. It has also been found to occur in different types of neurons however, the most common neurotransmitter involved in LTD is L-glutamate that acts on the NMDARs, AMPAR, KARs and metabotropic glutamate receptors (mGluRs). It can result from strong synaptic stimulation (as occurs e.g. in the cerebellar Purkinje cells) or from persistent weak synaptic stimulation (as in the hippocampus) resulting mainly from a decrease in postsynaptic AMPA receptor density, although a decrease in presynaptic neurotransmitter release may also play a role. Moreover, cerebellar LTD has been hypothesized to be important for motor learning and hippocampal LTD may be important for the clearing of old memory traces (Nicholls et al., 2008; Mallere et al., 2010). The main molecular mechanism underlying-LTD is the phosphorylation of AMPA glutamate receptors and their synaptic elimination (Ogasawara et al., 2008).

It is now commonly understood in the field of spine morphology that long lasting NMDAR-dependent LTD causes dendritic spine shrinkage, reduces number of synaptic AMPA receptors (Calabrese et al., 2014), possibly leading to synaptic dysfunction, contributing to decreased neuronal network function and impairment of learning and memory processes.

Additional text, specific for the AOP “Acetylcholinesterase inhibition leading to neurodegeneration”:

Seizures caused by cholinesterase dependent mechanisms result in an excess of glutamate release that activates the NMDA receptors. As a result, intracellular Ca2+ levels at the postsynaptic neuron can overload the calcium-control mechanisms, activating without control all the calcium-dependent enzymes (proteases, lipases…) (Deshpande et al., 2014; Garcia-Reyero et al., 2016). In cases of strong acetylcholinesterase inhibition of the CNS, the NMDAR overactivation initiated by cholinergic mechanisms can result, after the initial seizure activity (focal seizure), in the development of status epilepticus. This key event separates the initial toxicity, driven by cholinergic activity, from the secondary toxicity, which is cholinergic independent (McDonough and Shih, 1997).

### How it is Measured or Detected

*Methods that have been previously reviewed and approved by a recognized authority should be included in the Overview section above. All other methods, including those well established in the published literature, should be described here. Consider the following criteria when describing each method: 1. Is the assay fit for purpose? 2. Is the assay directly or indirectly (i.e. a surrogate) related to a key event relevant to the final adverse effect in question? 3. Is the assay repeatable? 4. Is the assay reproducible?*

No OECD methods are available to measure the activation state of NMDA receptors.

The measurement of the activation or the inhibition of NMDA receptors is done indirectly by recording the individual ion channels that are selective to Na+, K+ and Ca2+ by the patch clamp technique. This method relies on lack of measurable ion flux when NMDA ion channel is closed, whereas constant channel specific conductance is recorded at the open state of the receptor (Blanke and VanDongen, 2009). Furthermore, this method is based on the prediction that activation or inhibition of an ion channel results from an increase in the probability of being in the open or closed state, respectively (Ogdon and Stanfield, 2009; Zhao et al., 2009).

The whole-cell patch clamp recording techniques have also been used to study synaptically-evoked NMDA receptor-mediated excitatory or inhibitory postsynaptic currents (EPSCs and IPSCs, respectively) in brain slices and neuronal cells, allowing the evaluation of the activated or inhibited state of the receptor.

Microelectrode array (MEA) recordings are used to measure mainly spontaneous network activity of cultured neurons (Keefer et al., 2001, Gramowski et al., 2000 and Gopal, 2003; Johnstone et al., 2010). However, using specific agonists and antagonists of a receptor, including NMDAR, MEA technology can be used to measure evoked activity, including glutamatergic receptor function (Lantz et al., 2014). For example it has been shown that MEA-coupled neuronal cortical networks are very sensitive to pharmacological manipulation of the excitatory ionotropic glutamatergic transmission (Frega et al., 2012). MEAs can also be applied in higher throughput platforms to facilitate screening of numerous chemical compounds (McConnell et al., 2012).

Excessive excitability can be also measured directly by evaluating the level of the extracellular glutamate using the enzyme-based microelectrode arrays. This technology is capable of detecting glutamate in vivo, to assess the effectiveness of hyperexcitability modulators on glutamate release in brain slices. Using glutamate oxidase coated ceramic MEAs coupled with constant voltage amperometry, it is possible to measure resting glutamate levels and synaptic overflow of glutamate after K(+) stimulation in brain slices (Quintero et al., 2011).

Neuronal network function can be also measured using optical detection of neuronal spikes both in vivo and in vitro (Wilt et al., 2013).

Drebrin immunocytochemistry: drebrin, a major actin-filament-binding protein localized in mature dendritic spines is a target of calpain mediated proteolysis under excitotoxic conditions induced by the overactivation of NMDARs. In cultured rodent neurons, degradation of drebrin was confirmed by the detection of proteolytic fragments, as well as a reduction in the amount of full-length drebrin. The NMDA-induced degradation of drebrin in mature neurons occurres concomitantly with a loss of f-actin. Biochemical analyses using purified drebrin and calpain revealed that calpain degraded drebrin directly in vitro. These findings suggest that calpain-mediated degradation of drebrin is mediated by excitotoxicity, regardless of whether they are acute or chronic. Drebrin (A and E) regulates the synaptic clustering of NMDARs. Therefore, degradation of drebrin can be used as a readout for excitotoxicity induced by NMDAR overactivation. Degradation of drebrin can be evaluated quantitatively by Western blot analysis (mRNA evel) or by immunocytochemistry (at protein level) (Chimura et al., 2015: Sekino et al., 20069.

NMDAR overactivation-induced long lasting LTD can be measured by the dendritic spine shrinkage by quantification of cofilin and phospho-cofilin in neurons expressing eGFP and combined with immunocytochemical techniques (Calabrese et al., 2014).

### References

Blanke ML, VanDongen AMJ., Activation Mechanisms of the NMDA Receptor. In: Van Dongen AM, editor. Biology of the NMDA Receptor. Boca Raton (FL): CRC Press; 2009, Chapter 13. Available from: http://www.ncbi.nlm.nih.gov/books/NBK5274/.

Calabrese B, Saffin JM, Halpain S. Activity-dependent dendritic spine shrinkage and growth involve downregulation of cofilin via distinct mechanisms. PLoS One., 2014, 16;9(4):e94787.

Chimura T., Launey T., Yoshida N.,Calpain-Mediated Degradation of Drebrin by Excitotoxicity In vitro and In vivo PLOS ONE, 2015, |DOI:10.1371/journal.pone.0125119.

Deshpande, L. S., D. S. Carter, K. F. Phillips, R. E. Blair and R. J. DeLorenzo (2014), "Development of status epilepticus, sustained calcium elevations and neuronal injury in a rat survival model of lethal paraoxon intoxication", *NeuroToxicology* **44**: 17-26. DOI: 10.1016/j.neuro.2014.04.006.

Frega M, Pasquale V, Tedesco M, Marcoli M, Contestabile A, Nanni M, Bonzano L, Maura G, Chiappalone M., Cortical cultures coupled to micro-electrode arrays: a novel approach to perform in vitro excitotoxicity testing. Neurotoxicol Teratol. 2012: 34(1):116-27.

Garcia-Reyero, N., L. Escalon, E. Prats, M. Faria, A. M. V. M. Soares and D. Raldúa (2016), "Targeted Gene Expression in Zebrafish Exposed to Chlorpyrifos-Oxon Confirms Phenotype-Specific Mechanisms Leading to Adverse Outcomes", *Bulletin of Environmental Contamination and Toxicology* **96**(6): 707-713. DOI: 10.1007/s00128-016-1798-3.

Gopal K., Neurotoxic effects of mercury on auditory cortex networks growing on microelectrode arrays: a preliminary analysis. Neurotoxicol Teratol., 2003, 25: 69-76.

Gramowski A, Schiffmann D, Gross GW., Quantification of acute neurotoxic effects of trimethyltin using neuronal networks cultures on microelectrode arrays. Neurotoxicology, 2000, 21: 331-342.

Johnstone AFM, Gross GW, Weiss D, Schroeder O, Shafer TJ.,Use of microelectrode arrays for neurotoxicity testing in the 21st century Neurotoxicology, 2000, 31: 331-350.

Keefer E, Norton S, Boyle N, Talesa V, Gross G., Acute toxicity screening of novel AChE inhibitors using neuronal networks on microelectrode arrays. Neurotoxicology, 2001, 22: 3-12.

Lantz SR, Mack CM, Wallace K, Key EF, Shafer TJ, Casida JE. Glufosinate binds N-methyl-D-aspartate receptors and increases neuronal network activity in vitro. Neurotoxicology. 2014, 45:38-47.

Luscher C. and Malenka R.C., NMDA Receptor-Dependent Long-Term Potentiation and Long-Term Depression (LTP/LTD). Cold Spring Harb Perspect Biol., 2012, 4:a005710.

Malenka RC, Bear MF., LTP and LTD: An embarrassment of riches. Neuron, 2004, 44: 5–21.

Malleret G, Alarcon JM, Martel G, Takizawa S, Vronskaya S, Yin D, Chen IZ, Kandel ER, Shumyatsky GP., Bidirectional regulation of hippocampal long-term synaptic plasticity and its influence on opposing forms of memory". J Neurosci., 2010, 30 (10): 3813–25.

McConnell ER, McClain MA, Ross J, LeFew WR, Shafer TJ., Evaluation of multi-well microelectrode arrays for neurotoxicity screening using a chemical training set Neurotoxicology, 2012, 33: 1048-1057.

McDonough, J. H., Jr. and T. M. Shih (1997), "Neuropharmacological mechanisms of nerve agent-induced seizure and neuropathology", *Neurosci Biobehav Rev* **21**(5): 559-579.

Nicholls RE, Alarcon JM, Malleret G, Carroll RC, Grody M, Vronskaya S, Kandel ER., Transgenic mice lacking NMDAR-dependent LTD exhibit deficits in behavioral flexibility". Neuron, 2008, 58 (1): 104–17.

Ogasawara H, Doi T, Kawato M. Systems biology perspectives on cerebellar long-term depression. Neurosignals, 2008, 16 (4): 300–17.

Ogdon D, Stanfield P., Patch clamp techniques for single channel and whole-cell recording. Chapter 4, pages 53-78, (http://www.utdallas.edu/~tres/microelectrode/microelectrodes_ch04.pdf).

Paradiso MA, Bear MF, Connors BW., Neuroscience: exploring the brain. 2007, Hagerstwon, MD: Lippincott Williams & Wilkins. p. 718. ISBN 0-7817-6003-8.

Purves D., Neuroscience (4th ed.). Sunderland, Mass: Sinauer., 2008, pp. 197–200.ISBN 0-87893-697-1.

Sekino Y, Tanaka S, Hanamura K, Yamazaki H, Sasagawa Y, Xue Y, Hayashi K, Shirao T., Activation of N-methyl-D-aspartate receptor induces a shift of drebrin distribution: disappearance from dendritic spines and appearance in dendritic shafts. Mol Cell Neurosci. 2006, 31(3):493-504.

Quintero JE, Pomerleau F, Huettl P, Johnson KW, Offord J, Gerhardt GA. 2011. Methodology for rapid measures of glutamate release in rat brain slices using ceramic-based microelectrode arrays: basic characterization and drug pharmacology. Brain Res.2011, 1401:1-9.

Wilt BA, Fitzgerald JE, Schnitzer MJ., Photon shot noise limits on optical detection of neuronal spikes and estimation of spike timing. Biophys J. 2013, 8; 104(1):51-62.

Zhao Y, Inayat S, Dikin DA, Singer JH, Ruoff RS, Troy JB., Patch clamp techniques: review of the current state of art and potential contributions from nanoengineering. Proc. IMechE 222, Part N: J. Nanoengineering and Nanosystems, 2009, 149. DOI: 10.1243/17403499JNN149.

## Event: 389: Increased, Intracellular Calcium overload

### Short Name: Increased, Intracellular Calcium overload

### Event Component

| Process | Object | Action |
|---|---|---|

| Process | Object | Action |
|---|---|---|
| calcium ion transport | calcium ion | increased |

### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:48 - Binding of agonists to ionotropic glutamate receptors in adult brain causes excitotoxicity that mediates neuronal cell death, contributing to learning and memory impairment. | KeyEvent |
| Aop:281 - Acetylcholinesterase Inhibition Leading to Neurodegeneration | KeyEvent |
| Aop:464 - Calcium overload in dopaminergic neurons of the substantia nigra leading to parkinsonian motor deficits | KeyEvent |
| Aop:475 - Binding of chemicals to ionotropic glutamate receptors leads to impairment of learning and memory via loss of drebrin from dendritic spines of neurons | KeyEvent |
| Aop:535 - Binding and activation of GPER leading to learning and memory impairments | KeyEvent |
| Aop:556 - Decreased Na/K ATPase activity leading to heart failure | KeyEvent |
| Aop:558 - Phosphodiesterase inhibition leading to heart failure | KeyEvent |

### Biological Context

**Level of Biological Organization**

Cellular

### Cell term

| Cell term |
|---|
| eukaryotic cell |

### Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| zebrafish | Danio rerio | High | NCBI |
| Human, rat, mouse | Human, rat, mouse | High | NCBI |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| During brain development, adulthood and aging | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Mixed | Not Specified |

Please see KE Calcium influx, Decreasedin the AOP entitled *Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment of learning and memory abilities.*

Additional text, specific for the AOP “Acetylcholinesterase Inhibition leading to Neurodegeneration”:

Zebrafish have shown dysregulation in intracellular calcium ion levels following exposure to organophosphate compounds through similar mechanisms demonstrated in mammals (Faria et al. 2015).

### Key Event Description

NMDAR agonist binding results in increased intracellular calcium, whereas NMDAR antagonist binding results in decreased intracellular calcium levels. For the relevant paragraphs below please see AOP entitled *Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment of learning and memory abilities.*

**Biological state:** KE Calcium influx, Decreased

**Biological compartments:** KE Calcium influx, Decreased

**General role in biology:** KE Calcium influx, Decreased

The text specific for the AOP "ionotropic glutamatergic receptors and cognition" and "Acetylcholinesterase inhibition leading to neurodegeneration":

It is now well accepted that modest activation of NMDARs leading to modest increases in postsynaptic calcium are optimal for triggering LTD (Lledo et al. 1998; Bloodgood and Sabatin, 2007; Bloodgood et al. 2009), whereas much stronger activation of NMDARs leading to much larger increases in postsynaptic calcium are required to trigger LTP (Luscher and Malenka, 2012; Malenka 1994). Indeed, high-frequency stimulation causes a strong temporal summation of the excitatory postsynaptic potentials (EPSPs), and depolarization of the postsynaptic cell is sufficient to relieve the Mg2+ block of the NMDAR and allow a large amount of calcium to enter into the postsynaptic cells. Therefore, intra-cellular calcium is measured as a readout for evaluation NMDAR stimulation.

### How it is Measured or Detected

*Methods that have been previously reviewed and approved by a recognized authority should be included in the Overview section above. All other methods, including those well established in the published literature, should be described here. Consider the following criteria when describing each method: 1. Is the assay fit for purpose? 2. Is the assay directly or indirectly (i.e. a surrogate) related to a key event relevant to the final adverse effect in question? 3. Is the assay repeatable? 4. Is the assay reproducible?*

Please see KE Calcium influx, Decreasedin the AOP entitled: *Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment of learning and memory abilities.*

### References

Bloodgood BL, Sabatini BL., Nonlinear regulation of unitary synaptic signals by CaV2.3 voltage-sensitive calcium channels located in dendritic spines. Neuron, 2007, 53:249–260.

Bloodgood BL, Giessel AJ, Sabatini BL., Biphasic synaptic Ca influx arising from compartmentalized electrical signals in dendritic spines. PLoS Biol., 2009, 7: e1000190.

Faria, M., N. Garcia-Reyero, F. Padrós, P. J. Babin, D. Sebastián, J. Cachot, E. Prats, M. Arick Ii, E. Rial, A. Knoll-Gellida, G. Mathieu, F. Le Bihanic, B. L. Escalon, A. Zorzano, A. M. Soares and D. Raldúa (2015), "Zebrafish Models for Human Acute Organophosphorus Poisoning." *Sci Rep* **5**. DOI: 10.1038/srep15591.

Lledo PM, Zhang X, Sudhof TC, Malenka RC, Nicoll RA., Postsynaptic membrane fusion and long-term potentiation. Science, 1998, 279: 399–403.

Malenka RC. Synaptic plasticity in the hippocampus: LTP and LTD. Cell, 1994, 78: 535–538.

Luscher C. and Robert C. Malenka. NMDA Receptor-Dependent Long-Term Potentiation and Long-Term Depression (LTP/LTD). Cold Spring Harb Perspect Biol., 2012, 4: a005710.

## Event: 2078: Loss of drebrin

### Short Name: Loss of drebrin

### Event Component

| Process | Object | Action |
|---|---|---|
| postsynaptic actin cytoskeleton organization | drebrin | decreased |
| dendritic spine morphogenesis | actin cytoskeleton | decreased |
| regulation of actin-dependent ATPase activity | actin cytoskeleton | increased |

### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:475 - Binding of chemicals to ionotropic glutamate receptors leads to impairment of learning and memory via loss of drebrin from dendritic spines of neurons | KeyEvent |
| Aop:471 - Neuron defect induced early behavioral change | KeyEvent |

### Biological Context

**Level of Biological Organization**

Cellular

### Cell term

**Cell term**

neuron

### Organ term

**Organ term**

brain

## Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| Human, rat, mouse | Human, rat, mouse | High | NCBI |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| During brain development, adulthood and aging | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Unspecific | High |

This KE applies to mammals of both sexes, during neuronal development at any life stage (both immature and adult nervous system).

## Key Event Description

Glutamate induces the translocation of drebrin from dendritic spines to dendritic shafts, as reported in mature cultured rodent neurons and human iPS-derived neurons (Sekino et al. 2006; Mizui et al.2014; Lin et al. 2023). This "drebrin exodus" is a physiological process occurring at the initiation of synaptic plasticity, observed immediately after excitatory postsynapses receive electrical or chemical stimulation that induces long-term potentiation (LTP) or long-term depression (LTD) .

NMDA-induced excitotoxicity leads to the degradation of drebrin in mature cultured neurons from rodent hippocampus and cortex. This process is triggered by calcium influx and mediated by calpain activity.

Drebrin is an evolutionarily conserved actin-binding protein localized in dendritic spines. Overexpression of drebrin A in neurons enlarges dendritic spines and reduces spine motility, whereas down-regulation of drebrin A decreases the density and width of dendritic spines and inhibits the synaptic clustering of NMDA receptors (NMDARs).

Drebrin stabilizes actin filaments and plays a pivotal role in dendritic spine morphogenesis (Hayashi and Shirao, 1999; Takahashi et al., 2003; Takahashi et al., 2006).

During the initial stages of synaptic plasticity (either LTP or LTD), $Ca^{2+}$ influx through NMDA receptors triggers the translocation of drebrin from dendritic spines (Sekino et al., 2006).

Moreover, prolonged NMDA-induced excitotoxicity results in calpain-mediated degradation of drebrin both in vitro and in vivo.

## How it is Measured or Detected

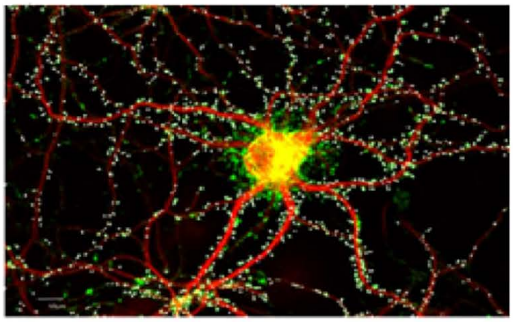
Loss of drebrin from dendritic spines can be detected by immunocytochemistry, ELISA or or Western blotting  in cultured human and rodent neurons and brain tissues (Counts et al., 2006; Ishizuka et al., 2014).

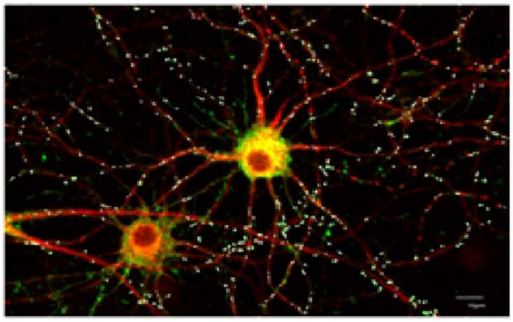
The twenty-one-day primary cultured neurons were prepared using frozen stock of dissociated hippocampal neurons (Koganezawa et al, 2023; Hanamura et al 2019). In brief, cells were cultured in multi well microplates with defined medium. Using cryopreservednhippocampal neurons reduces animal use.

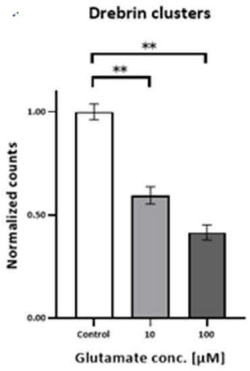
For enzyme-linked immunoassay, after the incubation of chemicals the extracts of neurons were quantified using ELISA kit for drebrin (ALzMEd, Inc.),

For immunocytochemistry, the cultured neurons were incubated with chemicals for 1 hour. After fixation, cultured neurons were immunostained with anti-drebrin and anti-MAP2 antibodies. The cluster density of drebrin along the dendrites was automatically quantified using high content analysis instruments (Hanamura et al, 2019, Mitsuoka et al, 2019).

Quantitative analysis of number of drebrin clusters








## References

Chronological order : newest first

Yamazaki H, Koganezawa N, Yokoo H, Sekino Y, Shirao T. Super-resolution imaging reveals the relationship between CaMKIIβ and drebrin within dendritic spines. Neurosci Res. 2024 Feb;199:30-35. doi: 10.1016/j.neures.2023.08.002. Epub 2023 Sep 1. PMID: 37659612.

Kajita Y, Kojima N, Shirao T. A lack of drebrin causes olfactory impairment. Brain Behav. 2024 Jan;14(1):e3354. doi: 10.1002/brb3.3354. PMID: 38376048; PMCID: PMC10757890.

Song L, Chen H, Qiao D, Zhang B, Guo F, Zhang Y, Wang C, Li S, Cui H. ZIP9 mediates the effects of DHT on learning, memory and hippocampal synaptic plasticity of male Tfm and APP/PS1 mice. Front Endocrinol (Lausanne). 2023 May 25;14:1139874. doi: 10.3389/fendo.2023.1139874. PMID: 37305050; PMCID: PMC10248430.

Lin W, Shiomoto S, Yamada S, Watanabe H, Kawashima Y, Eguchi Y, Muramatsu K, Sekino Y. Dendritic spine formation and synapse maturation in transcription factor-induced human iPSC-derived neurons. iScience. 2023 Feb 27;26(4):106285. doi: 10.1016/j.isci.2023.106285. Erratum in: iScience. 2023 Jul 20;26(8):107423. doi: 10.1016/j.isci.2023.107423. PMID: 37034988; PMCID: PMC10073939.

Koganezawa, N., Roppongi, R.T.,Sekino, Y., Tsutsui, I., Higa, A.,Shirao, T. Easy and Reproducible Low-Density Primary Culture using Frozen Stock of Embryonic Hippocampal Neurons. J. Vis. Exp. (191), e64872, doi:10.3791/64872 (2023).

Mitsuoka T, Hanamura K, Koganezawa N, Kikura-Hanajiri R, Sekino Y, Shirao T. “Assessment of NMDA receptor inhibition of phencyclidine analogues using a high-throughput drebrin immunocytochemical assay” J Pharmacol Toxicol Methods 2019 May 10:106583. doi: 10.1016/j.vascn.2019.106583.

Hanamura K, Koganezawa N, Kamiyama K, Tanaka N, Oka T, Yamamura M, Sekino Y, Shirao T. “High-content imaging analysis for detecting the loss of drebrin clusters along dendrites in cultured hippocampal neurons.” J Pharmacol Toxicol Methods. 2018 Sep - Oct;99:106607. doi: 10.1016/j.vascn.2019.106607.

Yamazaki H, Sasagawa Y, Yamamoto H, Bito H, Shirao T. CaMKIIβ is localized in dendritic spines as both drebrin-dependent and drebrin-independent pools. J Neurochem. 2018 Jul;146(2):145-159. doi: 10.1111/jnc.14449. Epub 2018 Jun 11. PMID: 29675826; PMCID: PMC6099455.

Cho C, MacDonald R, Shang J, Cho MJ, Chalifour LE, Paudel HK. Early growth response-1-mediated down-regulation of drebrin correlates with loss of dendritic spines. J Neurochem. 2017 Jul;142(1):56-73. doi: 10.1111/jnc.14031. Epub 2017 Apr 26. PMID: 28369888.

Liu Y, Xu YF, Zhang L, Huang L, Yu P, Zhu H, Deng W, Qin C. Effective expression of Drebrin in hippocampus improves cognitive function and alleviates lesions of Alzheimer's disease in APP (swe)/PS1 (ΔE9) mice. CNS Neurosci Ther. 2017 Jul;23(7):590-604. doi: 10.1111/cns.12706. Epub 2017 Jun 8. PMID: 28597477; PMCID: PMC6492767.

Sekino Y, Koganezawa N, Mizui T, Shirao T. Role of Drebrin in Synaptic Plasticity. Adv Exp Med Biol. 2017;1006:183-201. doi: 10.1007/978-4-431-56550-5_11. PMID: 28865021.

Puspitasari A, Koganezawa N, Ishizuka Y, Kojima N, Tanaka N, Nakano T, Shirao T. X Irradiation Induces Acute Cognitive Decline via Transient Synaptic Dysfunction. Radiat Res. 2016 Apr;185(4):423-30. doi: 10.1667/RR14236.1. Epub 2016 Mar 29. PMID: 27023259.

Chimura T, Launey T, Yoshida N (2015) Calpain-Mediated Degradation of Drebrin by Excitotoxicity In vitro and In vivo. PLoS ONE 10(4): e0125119. doi:10.1371/journal.pone.0125119

Jung G, Kim EJ, Cicvaric A, Sase S, Gröger M, Höger H, Sialana FJ, Berger J, Monje FJ, Lubec G. Drebrin depletion alters neurotransmitter receptor levels in protein complexes, dendritic spine morphogenesis and memory-related synaptic plasticity in the mouse hippocampus.

J Neurochem. 2015 Jul;134(2):327-39. doi: 10.1111/jnc.13119. Epub 2015 Apr 29. PMID: 25865831.

Ishizuka Y, Shimizu H, Takagi E, Kato M, Yamagata H, Mikuni M, Shirao T. Histone deacetylase mediates the decrease in drebrin cluster density induced by amyloid beta oligomers. Neurochem Int. 2014 Oct;76:114-21. doi: 10.1016/j.neuint.2014.07.005. Epub 2014 Jul 21. PMID: 25058791.

Mizui T, Sekino Y, Yamazaki H, Ishizuka Y, Takahashi H, Kojima N, Kojima M, Shirao T. Myosin II ATPase activity mediates the long-term potentiation-induced exodus of stable F-actin bound by drebrin A from dendritic spines. PLoS One. 2014 Jan 22;9(1):e85367. doi: 10.1371/journal.pone.0085367. PMID: 24465547; PMCID: PMC3899004.

Roppongi RT, Kojima N, Hanamura K, Yamazaki H, Shirao T. Selective reduction of drebrin and actin in dendritic spines of hippocampal neurons by activation of 5-HT(2A) receptors. Neurosci Lett. 2013 Jun 28;547:76-81. doi: 10.1016/j.neulet.2013.04.061. Epub 2013 May 14. PMID: 23684573.

Kojima N, Hanamura K, Yamazaki H, Ikeda T, Itohara S, Shirao T. Genetic disruption of the alternative splicing of drebrin gene impairs context-dependent fear learning in adulthood. Neuroscience. 2010 Jan 13;165(1):138-50. doi: 10.1016/j.neuroscience.2009.10.016. Epub 2009 Oct 24. PMID: 19837137.

Aoki C, Kojima N, Sabaliauskas N, Shah L, Ahmed TH, Oakford J, Ahmed T, Yamazaki H, Hanamura K, Shirao T. Drebrin a knockout eliminates the rapid form of homeostatic synaptic plasticity at excitatory synapses of intact adult cerebral cortex. J Comp Neurol. 2009 Nov 1;517(1):105-21. doi: 10.1002/cne.22137. PMID: 19711416; PMCID: PMC2839874.

Ivanov A, Esclapez M, Ferhat L. Role of drebrin A in dendritic spine plasticity and synaptic function: Implications in neurological disorders. Commun Integr Biol. 2009 May;2(3):268-70. doi: 10.4161/cib.2.3.8166. PMID: 19641748; PMCID: PMC2717538.

Lacor PN, Buniel MC, Furlow PW, Clemente AS, Velasco PT, Wood M, Viola KL, Klein WL. Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. J Neurosci. 2007 Jan 24;27(4):796-807. doi: 10.1523/JNEUROSCI.3501-06.2007. PMID: 17251419; PMCID: PMC6672917.

Takahashi H, Mizui T, Shirao T. Down-regulation of drebrin A expression suppresses synaptic targeting of NMDA receptors in developing hippocampal neurones. J Neurochem. 2006 Apr;97 Suppl 1:110-5. doi: 10.1111/j.1471-4159.2005.03536.x. PMID: 16635259.

Sekino Y, Tanaka S, Hanamura K, Yamazaki H, Sasagawa Y, Xue Y, Hayashi K, Shirao T. (2006) Activation of N-methyl-D-aspartate receptor induces a shift of drebrin distribution: disappearance from dendritic spines and appearance in dendritic shafts. Mol Cell Neurosci.Mar;31(3):493-504. doi: 10.1016/j.mcn.2005.11.003. Epub 2005 Dec 20. PMID: 16368245.

Kobayashi R, Sekino Y, Shirao T, Tanaka S, Ogura T, Inada K, Saji M. Antisense knockdown of drebrin A, a dendritic spine protein, causes stronger preference, impaired pre-pulse inhibition, and an increased sensitivity to psychostimulant. Neurosci Res. 2004 Jun;49(2):205-17. doi: 10.1016/j.neures.2004.02.014. PMID: 15140563.

Aoki C, Fujisawa S, Mahadomrongkul V, Shah PJ, Nader K, Erisir A. NMDA receptor blockade in intact adult cortex increases trafficking of NR2A subunits into spines, postsynaptic densities, and axon terminals. Brain Res. 2003 Feb 14;963(1-2):139-49. doi: 10.1016/s0006-8993(02)03962-8. PMID: 12560119.

Shim KS, Lubec G. Drebrin, a dendritic spine protein, is manifold decreased in brains of patients with Alzheimer's disease and Down syndrome. Neurosci Lett. 2002 May 24;324(3):209-12. doi: 10.1016/s0304-3940(02)00210-0. PMID: 12009525.

Tomidokoro Y, Harigaya Y, Matsubara E, Ikeda M, Kawarabayashi T, Shirao T, Ishiguro K, Okamoto K, Younkin SG, Shoji M. Brain Abeta amyloidosis in APPsw mice induces accumulation of presenilin-1 and tau. J Pathol. 2001 Aug;194(4):500-6. doi: 10.1002/path.897. PMID: 11523060.

Harigaya Y, Shoji M, Shirao T, Hirai S. Disappearance of actin-binding protein, drebrin, from hippocampal synapses in Alzheimer's disease. J Neurosci Res. 1996 Jan 1;43(1):87-92. doi: 10.1002/jnr.490430111. PMID: 8838578.

## Event: 2242: Abnormality, dendritic spine morphology

### Short Name: Dendritic spine abnormality

### Event Component

| Process | Object | Action |
|---|---|---|
| negative regulation of synaptic plasticity | postsynaptic actin cytoskeleton | pathological |
| Dementia | drebrin | decreased |
| long term synaptic depression | drebrin | decreased |
| abnormal long term potentiation | drebrin | increased |

### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:475 - Binding of chemicals to ionotropic glutamate receptors leads to impairment of learning and memory via loss of drebrin from dendritic spines of neurons | KeyEvent |

### Biological Context

**Level of Biological Organization**

Cellular

## Cell term

**Cell term**

neuron

## Organ term

**Organ term**

brain

## Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| Human, rat, mouse | Human, rat, mouse | High | NCBI |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| During brain development, adulthood and aging | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Unspecific | High |

This KE applies to mammals of both sexes, during neuronal development at any life stage (both immature and adult nervous system).

## Key Event Description

Abnormalities in dendritic spines have been implicated in a wide range of psychiatric and neurological disorders, with initial discoveries stemming from Golgi staining of postmortem brains. These spine pathologies have been observed across various conditions, including schizophrenia, autism spectrum disorders (ASD), Alzheimer's disease (AD), bipolar disorder, Down syndrome, and epilepsy.Alzheimer's disease brains exhibit reduced synapse density and dendritic spine loss in the cortex and hippocampus, with greater spine loss associated with lower mental statusThis synaptic pathology is considered one of the earliest features of AD, occurring prior to neuronal loss. The shared feature of spine pathology across these disorders suggests that dendritic spines may serve as a common substrate for many brain disorders involving deficits in information processing and neuronal connectivity.

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## How it is Measured or Detected

Drebrin immunocytochemistry can be used to measure the number of drebrin clusters.

## References

Results from the National Center for Biotechnology Information (NCBI) at the U.S. National Library of Medicine (NLM).

**Search:** Dendritic Spine abnormality

Marin-Padilla M. Pyramidal cell abnormalities in the motor cortex of a child with Down's syndrome. A Golgi study. J Comp Neurol. 1976 May 1;167(1):63-81. doi: 10.1002/cne.901670105. PMID: 131810.

Gonatas NK, Moss A. Pathologic axons and synapses in human neuropsychiatric disorders. Hum Pathol. 1975 Sep;6(5):571-82. doi: 10.1016/s0046-8177(75)80042-6. PMID: 170188.

Marin-Padilla M. Abnormal neuronal differentiation (functional maturation) in mental retardation. Birth Defects Orig Artic Ser. 1975;11(7):133-53. PMID: 764896.

Purpura DP. Normal and aberrant neuronal development in the cerebral cortex of human fetus and young infant. UCLA Forum Med Sci. 1975;(18):141-69. doi: 10.1016/b978-0-12-139050-1.50014-8. PMID: 128168.

Purpura DP. Dendritic spine "dysgenesis" and mental retardation. Science. 1974 Dec 20;186(4169):1126-8. doi: 10.1126/science.186.4169.1126. PMID: 4469701.

Search : decrease of drebrin

Xie MJ, Yagi H, Iguchi T, Yamazaki H, Hanamura K, Matsuzaki H, Shirao T, Sato M. Phldb2 is essential for regulating hippocampal

dendritic spine morphology through drebrin in an adult-type isoform-specific manner. Neurosci Res. 2022 Dec;185:1-10. doi: 10.1016/j.neures.2022.09.010. Epub 2022 Sep 23. PMID: 36162735.

## Event: 1944: Synaptic dysfunction

### Short Name: Dysfunctional synapses

### Event Component

| Process | Object | Action |
|---|---|---|
| decreased neurotransmitter release | CNS neuron (sensu Vertebrata) | decreased |
| increased synaptic depression | CNS neuron (sensu Vertebrata) | increased |

### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:429 - A cholesterol/glucose dysmetabolism initiated Tau-driven AOP toward memory loss (AO) in sporadic Alzheimer's Disease with plausible MIE's plug-ins for environmental neurotoxicants | KeyEvent |
| Aop:475 - Binding of chemicals to ionotropic glutamate receptors leads to impairment of learning and memory via loss of drebrin from dendritic spines of neurons | KeyEvent |
| Aop:471 - Neuron defect induced early behavioral change | KeyEvent |
| Aop:652 - Inhibition of brain insulin-degrading enzyme (IDE) leads to cognitive decline (adult) | KeyEvent |

### Biological Context

**Level of Biological Organization**

Organ

### Organ term

| Organ term |
|---|
| principal neuronal circuit |

### Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| Human, rat, mouse | Human, rat, mouse | High | NCBI |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Unspecific | High |

Species applicability includes humans, using clinical assessments like neuroimaging, EEG, and cognitive tests; animal models (e.g., rodents) employing electrophysiological, behavioral, and biochemical assays; and cultured neuronal cells for molecular-level studies. Life-stage applicability covers developmental stages for neurodevelopmental disorders, adulthood for psychiatric and neurodegenerative diseases, and elderly populations for age-related cognitive decline. Experimental system applicability spans in vitro models for cellular mechanisms, ex vivo brain slices for electrophysiological studies, and in vivo animal models for behavioral and systemic analysis. Disease applicability involves neurological and psychiatric conditions such as Alzheimer's, Parkinson's, schizophrenia, depression, epilepsy, and autism spectrum disorders. Clearly defining these domains ensures accurate interpretation, facilitates translational research, and supports targeted therapeutic development for various neurological and psychiatric disorders.

### Key Event Description

Synaptic dysfunction refers to the impairment or disruption of communication between neurons at synapses. It occurs when neurotransmitter release, receptor function, or synaptic structure is altered or damaged, leading to impaired signaling. Such dysfunction can contribute to various neurological and psychiatric conditions, including Alzheimer's disease, Parkinson's disease, depression, schizophrenia, and autism spectrum disorders.

### How it is Measured or Detected

- **Electrophysiology (Patch Clamp, Field Potential Recording)**: Measures neurotransmition efficiency and receptor function by currents recordings. Evaluate synaptic plasticity, such as LTP and LTD
- **Microdialysis or HPLC (High-Performance Liquid Chromatography)**: Quantifies neurotransmitter levels directly.
- **Western Blot, ELISA**: Evaluate proteins expression.
- **Immunohistochemistry and imagings :** Evaluate expression and locarization for pre- and post synaptic proteins, mitochondrial imaging, infkammation and immune responses using microscopies.
- **Behavioral Tests (e.g., Morris Water Maze, Novel Object Recognition)**: Evaluate cognitive functions related to synaptic plasticity.
- **Morphlogical changes: Electron Microscopy (EM)**: Visualize synaptic ultrastructure. **Confocal or Two-photon Microscopy**: Observe dendritic spine morphology and density changes. **Golgi Staining**: Analyze dendritic spine density and morphology.
- **MRI, PET EEG**

### References

Jocelyn R Barnes , Bandhan Mukherjee , Ben C Rogers , Firoozeh Nafar , Madeline Gosse , Matthew P Parsons.,The Relationship Between Glutamate Dynamics and Activity-Dependent Synaptic Plasticity. J Neurosci. 2020 Apr 1;40(14):2793-2807.

## Event: 386: Decrease of neuronal network function

### Short Name: Neuronal network function, Decreased

### Event Component

| Process | Object | Action |
|---|---|---|
| synaptic signaling | | decreased |

### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:13 - Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment of learning and memory abilities | KeyEvent |
| Aop:78 - Nicotinic acetylcholine receptor activation contributes to abnormal role change within the worker bee caste leading to colony death failure 1 | KeyEvent |
| Aop:90 - Nicotinic acetylcholine receptor activation contributes to abnormal roll change within the worker bee caste leading to colony loss/failure 2 | KeyEvent |
| Aop:54 - Inhibition of Na+/I- symporter (NIS) leads to learning and memory impairment | KeyEvent |
| Aop:17 - Binding of electrophilic chemicals to SH(thiol)-group of proteins and /or to seleno-proteins involved in protection against oxidative stress during brain development leads to impairment of learning and memory | KeyEvent |
| Aop:405 - Organo-Phosphate Chemicals induced inhibition of AChE leading to impaired cognitive function | KeyEvent |
| Aop:429 - A cholesterol/glucose dysmetabolism initiated Tau-driven AOP toward memory loss (AO) in sporadic Alzheimer's Disease with plausible MIE's plug-ins for environmental neurotoxicants | KeyEvent |
| Aop:501 - Excessive iron accumulation leading to neurological disorders | KeyEvent |
| Aop:475 - Binding of chemicals to ionotropic glutamate receptors leads to impairment of learning and memory via loss of drebrin from dendritic spines of neurons | KeyEvent |
| Aop:522 - Estrogen antagonism leading to increased risk of autism-like behavior | KeyEvent |
| Aop:533 - Retinoic acid receptor antagonism during neurodevelopment leading to impaired learning and memory | KeyEvent |
| Aop:610 - Decreased thyroid hormone levels in the brain regulated via transport, metabolism and TR activation leading to decreased cognition and motor function | KeyEvent |
| Aop:636 - Increase in reactive oxygen species (ROS) leading to human amyotrophic lateral sclerosis (ALS) | KeyEvent |

### Biological Context

**Level of Biological Organization**

Organ

### Organ term

**Organ term**

brain

### Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| humans | Homo sapiens | High | NCBI |
| rat | Rattus norvegicus | High | NCBI |
| mice | Mus sp. | High | NCBI |
| cat | Felis catus | High | NCBI |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| During brain development | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Mixed | High |

In vitro studies in brain slices applying electrophysiological techniques showed significant variability among species (immature rats, rabbits and kittens) related to synaptic latency, duration, amplitude and efficacy in spike initiation (reviewed in Erecinska et al., 2004).

### Key Event Description

**Biological state:** There are striking differences in neuronal network formation and function among the developing and mature brain. The developing brain shows a slow maturation and a transient passage from spontaneous, long-duration action potentials to synaptically-triggered, short-duration action potentials.

Furthermore, at this precise developmental stage the neuronal network is characterised by "hyperexcitability", which is related to the increased number of local circuit recurrent excitatory synapses and the lack of γ-amino-butyric acid A (GABAA)-mediated inhibitory function that appears much later. This "hyperexcitability" disappears with maturation when pairing of the pre- and postsynaptic partners occurs and synapses are formed generating population of postsynaptic potentials and population of spikes followed by developmental GABA switch. Glutamatergic neurotransmission is dominant at early stages of development and NMDA receptor-mediated synaptic currents are far more times longer than those in maturation, allowing more calcium to enter the neurons. The processes that are involved in increased calcium influx and the subsequent intracellular events seem to play a critical role in establishment of wiring of neural circuits and strengthening of synaptic connections during development (reviewed in Erecinska et al., 2004). Neurons that do not receive glutaminergic stimulation are undergoing developmental apoptosis.

During the neonatal period, the brain is subject to profound alterations in neuronal circuitry due to high levels of synaptogenesis and gliogenesis. For example, in neuroendocrine regions such as the preoptic area-anterior hypothalamus (POA-AH), the site of gonadotropin-releasing hormone (GnRH) system is developmentally regulated by glutamatergic neurons. The changes in the expression of the N-methyl-D-aspartate (NMDA) receptor subunits NR1 and NR2B system begin early in postnatal development, before the onset of puberty, thereby playing a role in establishing the appropriate environment for the subsequent maturation of GnRH neurons (Adams et al., 1999).

**Biological compartments:** Neural network formation and function happen in all brain regions but it appears to onset at different time points of development (reviewed in Erecinska et al., 2004). Glutamatergic neurotransmission in hippocampus is poorly developed at birth. Initially, NMDA receptors play important role but the vast majority of these premature glutamatergic synapses are "silent" possibly due to delayed development of hippocampal AMPA receptors. In contrast, in the cerebral cortex the maturation of excitatory glutamatergic neurotransmission happens much earlier. The "silent" synapses disappear by PND 7-8 in both brain regions mentioned above.

There is strong evidence suggesting that NMDA receptor subunit composition controls synaptogenesis and synapse stabilization (Gambrill and Barria, 2011). It is established fact that during early postnatal development in the rat hippocampus, synaptogenesis occurs in parallel with a developmental switch in the subunit composition of NMDA receptors from NR2B to NR2A. It is suggested that early expression of NR2A in organotypic hippocampal slices reduces the number of synapses and the volume and dynamics of spines. In contrast, overexpression of NR2B does not affect the normal number and growth of synapses. However, it does increase spine motility, adding and retracting spines at a higher rate. The C terminus of NR2B, and specifically its ability to bind CaMKII, is sufficient to allow proper synapse formation and maturation. Conversely, the C terminus of NR2A was sufficient to stop the development of synapse number and spine growth. These results indicate that the ratio of synaptic NR2B over NR2A controls spine motility and synaptogenesis, and suggest a structural role for the intracellular C terminus of NR2 in recruiting the signalling and scaffolding molecules necessary for proper synaptogenesis. Interestingly, it was found that genetic deletion of NR3A accelerates glutamatergic synaptic transmission, as measured by AMPAR-mediated postsynaptic currents recorded in hippocampal CA1. Consistent, the deletion of NR3A accelerates the expression of the glutamate receptor subunits NR1, NR2A, and GluR1 sugesting that glutamatergic synapse maturation is critically dependent upon activation of NMDA-type glutamate receptors (Henson et al., 2012).

**General role in biology:** The development of neuronal networks can be distinguished into two phases: an early 'establishment' phase of neuronal connections, where activity-dependent and independent mechanisms could operate, and a later 'maintenance' phase, which appears to be controlled by neuronal activity (Yuste and Sur, 1999). These neuronal networks facilitate information flow that is necessary to produce complex behaviors, including learning and memory (Mayford et al., 2012).

### How it is Measured or Detected

*Methods that have been previously reviewed and approved by a recognized authority should be included in the Overview section above. All other methods, including those well established in the published literature, should be described here. Consider the following criteria when describing each method: 1. Is the assay fit for purpose? 2. Is the assay directly or indirectly (i.e. a surrogate) related to a key event relevant to the final adverse effect in question? 3. Is the assay repeatable? 4. Is the assay reproducible?*

**In vivo:** The recording of brain activity by using electroencephalography (EEG), electrocorticography (ECoG) and local field potentials (LFP) assists towards the collection of signals generated by multiple neuronal cell networks. Advances in computer technology have allowed quantification of the EEG and expansion of quantitative EEG (qEEG) analysis providing a sensitive tool for time-course studies of different compounds acting on neuronal networks' function (Binienda et al., 2011). The number of excitatory or inhibitory synapses can

be functionally studied at an electrophysiological level by examining the contribution of glutamatergic and GABAergic synaptic inputs. The number of them can be determined by variably clamping the membrane potential and recording excitatory and inhibitory postsynaptic currents (EPSCs or IPSCs) (Liu, 2004).

**In vitro:** Microelectrode array (MEA) recordings are also used to measure electrical activity in cultured neurons (Keefer et al., 2001, Gramowski et al., 2000; Gopal, 2003; Johnstone et al., 2010). MEAs can be applied in high throughput platforms to facilitate screening of numerous chemical compounds (McConnell et al., 2012). Using selective agonists and antagonists of different classes of receptors their response can be evaluated in a quantitative manner (Novellino et al., 2011; Hogberg et al., 2011).

Patch clamping technique can also be used to measure neuronal network activity.In some cases, if required, planar patch clamping technique can also be used to measure neuronal networks activity (e.g., Bosca et al., 2014).

### References


Adams MM, Flagg RA, Gore AC., Perinatal changes in hypothalamic N-methyl-D-aspartate receptors and their relationship to gonadotropin-releasing hormone neurons. Endocrinology. 1999 May;140(5):2288-96.

Binienda ZK, Beaudoin MA, Thorn BT, Ali SF. (2011) Analysis of electrical brain waves in neurotoxicology: γ-hydroxybutyrate. Curr Neuropharmacol. 9: 236-239.

Bosca, A., M. Martina, and C. Py (2014) Planar patch clamp for neuronal networks--considerations and future perspectives. Methods Mol Biol, 2014. 1183: p. 93-113.

Erecinska M, Cherian S, Silver IA. (2004) Energy metabolism in mammalian brain during development. Prog Neurobiol. 73: 397-445.

Gambrill AC, Barria A. NMDA receptor subunit composition controls synaptogenesis and synapse stabilization. Proc Natl Acad Sci U S A. 2011:108(14):5855-60.

Gopal K. (2003) Neurotoxic effects of mercury on auditory cortex networks growing on microelectrode arrays: a preliminary analysis. Neurotoxicol Teratol. 25: 69-76.

Gramowski A, Schiffmann D, Gross GW. (2000) Quantification of acute neurotoxic effects of trimethyltin using neuronal networks cultures on microelectrode arrays. Neurotoxicology 21: 331-342.

Henson MA, Larsen RS, Lawson SN, Pérez-Otaño I, Nakanishi N, Lipton SA, Philpot BD. (2012) Genetic deletion of NR3A accelerates glutamatergic synapse maturation. PLoS One. 7(8).

Hogberg HT, Sobanski T, Novellino A, Whelan M, Weiss DG, Bal-Price AK. (2011) Application of micro-electrode arrays (MEAs) as an emerging technology for developmental neurotoxicity: evaluation of domoic acid-induced effects in primary cultures of rat cortical neurons. Neurotoxicology 32: 158-168.

Johnstone AFM, Gross GW, Weiss D, Schroeder O, Shafer TJ. (2010) Use of microelectrode arrays for neurotoxicity testing in the 21st century Neurotoxicology 31: 331-350.

Keefer E, Norton S, Boyle N, Talesa V, Gross G. (2001) Acute toxicity screening of novel AChE inhibitors using neuronal networks on microelectrode arrays. Neurotoxicology 22: 3-12.

Liu G. (2004) Local structural balance and functional interaction of excitatory and inhibitory synapses in hippocampal dendrites. Nat Neurosci. 7: 373-379.

Mayford M, Siegelbaum SA, Kandel ER. (2012) Synapses and memory storage. Cold Spring Harb Perspect Biol. 4. pii: a005751.

McConnell ER, McClain MA, Ross J, LeFew WR, Shafer TJ. (2012) Evaluation of multi-well microelectrode arrays for neurotoxicity screening using a chemical training set. Neurotoxicology 33: 1048-1057.

Novellino A, Scelfo B, Palosaari T, Price A, Sobanski T, Shafer TJ, Johnstone AF, Gross GW, Gramowski A, Schroeder O, Jügelt K, Chiappalone M, Benfenati F, Martinoia S, Tedesco MT, Defranchi E, D'Angelo P, Whelan M. (2011) Development of micro-electrode array based tests for neurotoxicity: assessment of interlaboratory reproducibility with neuroactive chemicals. Front Neuroeng. 4: 4.

Yuste R, Peinado A, Katz LC. (1992) Neuronal domains in developing neocortex. Science 257: 665-669.


## List of Adverse Outcomes in this AOP

**Event: 341: Impairment, Learning and memory**

### Short Name: Impairment, Learning and memory

### Event Component

| Process | Object | Action |
|---|---|---|
| learning | | decreased |
| memory | | decreased |

### AOPs Including This Key Event

| AOP ID and Name | Event Type |
|---|---|
| Aop:13 - Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment of learning and memory abilities | AdverseOutcome |

| AOP ID and Name | Event Type |
|---|---|
| Aop:48 - Binding of agonists to ionotropic glutamate receptors in adult brain causes excitotoxicity that mediates neuronal cell death, contributing to learning and memory impairment. | AdverseOutcome |
| Aop:54 - Inhibition of Na+/I- symporter (NIS) leads to learning and memory impairment | AdverseOutcome |
| Aop:77 - Nicotinic acetylcholine receptor activation contributes to abnormal foraging and leads to colony death/failure 1 | KeyEvent |
| Aop:78 - Nicotinic acetylcholine receptor activation contributes to abnormal role change within the worker bee caste leading to colony death failure 1 | KeyEvent |
| Aop:87 - Nicotinic acetylcholine receptor activation contributes to abnormal foraging and leads to colony loss/failure | KeyEvent |
| Aop:88 - Nicotinic acetylcholine receptor activation contributes to abnormal foraging and leads to colony loss/failure via abnormal role change within caste | KeyEvent |
| Aop:89 - Nicotinic acetylcholine receptor activation followed by desensitization contributes to abnormal foraging and directly leads to colony loss/failure | KeyEvent |
| Aop:90 - Nicotinic acetylcholine receptor activation contributes to abnormal roll change within the worker bee caste leading to colony loss/failure 2 | KeyEvent |
| Aop:12 - Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development leads to neurodegeneration with impairment in learning and memory in aging | AdverseOutcome |
| Aop:99 - Histamine (H2) receptor antagonism leading to reduced survival | KeyEvent |
| Aop:17 - Binding of electrophilic chemicals to SH(thiol)-group of proteins and /or to seleno-proteins involved in protection against oxidative stress during brain development leads to impairment of learning and memory | AdverseOutcome |
| Aop:475 - Binding of chemicals to ionotropic glutamate receptors leads to impairment of learning and memory via loss of drebrin from dendritic spines of neurons | AdverseOutcome |
| Aop:483 - Deposition of Energy Leading to Learning and Memory Impairment | AdverseOutcome |
| Aop:490 - Co-activation of IP3R and RyR leads to reduced IQ and increased socio-economic burden through non-cholinergic mechanisms | AdverseOutcome |
| Aop:499 - Activation of MEK-ERK1/2 leads to deficits in learning and cognition via disrupted neurotransmitter release | AdverseOutcome |
| Aop:500 - Activation of MEK-ERK1/2 leads to deficits in learning and cognition via ROS and apoptosis | AdverseOutcome |
| Aop:520 - Retinoic acid receptor agonism during neurodevelopment leading to impaired learning and memory | AdverseOutcome |
| Aop:525 - Reduced oligodendrocyte differentiation during neurodevelopment leading to impaired learning and memory | AdverseOutcome |
| Aop:533 - Retinoic acid receptor antagonism during neurodevelopment leading to impaired learning and memory | AdverseOutcome |
| Aop:535 - Binding and activation of GPER leading to learning and memory impairments | AdverseOutcome |
| Aop:610 - Decreased thyroid hormone levels in the brain regulated via transport, metabolism and TR activation leading to decreased cognition and motor function | AdverseOutcome |

## Biological Context

**Level of Biological Organization**

Individual

## Domain of Applicability

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| human | Homo sapiens | High | NCBI |
| rat | Rattus norvegicus | High | NCBI |
| fruit fly | Drosophila melanogaster | High | NCBI |
| zebrafish | Danio rerio | High | NCBI |
| gastropods | Physa heterostropha | High | NCBI |
| mouse | Mus musculus | High | NCBI |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| During brain development | High |
| Adult, reproductively mature | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|

| Sex | Evidence |
|---|---|
| Mixed | High |

Basic forms of learning behavior such as habituation have been found in many taxa from worms to humans (Alexander, 1990). More complex cognitive processes such as executive function likely reside only in higher mammalian species such as non-human primates and humans. Recently, larval zebrafish has also been suggested as a model for the study of learning and memory (Roberts et al., 2013).

**Life stage applicability**: This key event is applicable to various life stages such as during brain development and maturity (Hladik & Tapio, 2016).

**Sex applicability:** This key event is not sex specific (Cekanaviciute et al., 2018), although sex-dependent cognitive outcomes have been recently ; Parihar et al., 2020).

**Evidence for perturbation by a prototypic stressor:** Current literature provides ample evidence of impaired learning and memory being induced by ionizing radiation (Cekanaviciute et al., 2018; Hladik & Tapio, 2016).

### Key Event Description

(Adapted from KE: 341 - in blue)

Learning can be defined as the process by which new information is acquired to establish knowledge by systematic study or by trial and error (Ono, 2009). Two types of learning are considered in neurobehavioral studies: a) associative learning and b) non- associative learning. Associative learning is based on making associations between different events. In associative learning, a subject learns the relationship among two different stimuli or between the stimulus and the subject's behavior. On the other hand, non-associative learning can be defined as an alteration in the behavioral response that occurs over time in response to a single type of stimulus. Habituation and sensitization are some examples of non-associative learning.

The memory formation requires acquisition, retention and retrieval of information in the brain, which is characterized by the non-conscious recall of information (Ono, 2009). There are three main categories of memory, including sensory memory, short-term or working memory (up to a few hours) and long-term memory (up to several days or even much longer).

Learning and memory depend upon the coordinated action of different brain regions and neurotransmitter systems constituting functionally integrated neural networks (D'Hooge and DeDeyn, 2001). Among the many brain areas engaged in the acquisition of, or retrieval of, a learned event, the hippocampal-based memory systems have received the most study. For example, the hippocampus has been shown to be critical for spatial-temporal memory, visio-spatial memory, verbal and narrative memory, and episodic and autobiographical memory (Burgess et al., 2000; Vorhees and Williams, 2014). However, there is substantial evidence that fundamental learning and memory functions are not mediated by the hippocampus alone but require a network that includes, in addition to the hippocampus, anterior thalamic nuclei, mammillary bodies cortex, cerebellum and basal ganglia (Aggleton and Brown, 1999; Doya, 2000; Mitchell et al., 2002, Toscano and Guilarte, 2005; Gilbert et al., 2006, 2016). Thus, damage to variety of

brain structures can potentially lead to impairment of learning and memory. The main learning areas and pathways are similar in rodents and primates, including man (Eichenbaum, 2000; Stanton and Spear, 1990). While the prefrontal cortex and frontostriatal neural circuits have been identified as the primary sites of higher-order cognition in vertebrates, invertebrates utilize paired mushroom bodies, shown to contain ~300,000 neurons in honey bees (Menzel, 2012; Puig et al., 2014).

For the purposes of this KE (AO), impaired learning and memory is defined as an organism's inability to establish new associative or non-associative relationships, or sensory, short-term or long-term memories which can be measured using different behavioral tests described below.

### How it is Measured or Detected

In laboratory animals: in rodents, a variety of tests of learning and memory have been used to probe the integrity of hippocampal function. These include tests of spatial learning like the radial arm maze (RAM), the Barnes maze, Hebb-Williams maze, passive avoidance and Spontaneous alternation and most commonly, the Morris water maze (MWM). Test of novelty such as novel object recognition, and fear based context learning are also sensitive to hippocampal disruption. Finally, trace fear conditioning which incorporates a temporal component upon traditional amygdala-based fear learning engages the hippocampus. A brief description of these tasks follows.

RAM, Barnes, MWM, Hebb-Williams maze are examples of spatial tasks, animals are required to learn the location of a food reward (RAM); an escape hole to enter a preferred dark tunnel from a brightly lit open field area (Barnes maze), or a hidden platform submerged below the surface of the water in a large tank of water (MWM) (Vorhees and Williams, 2014). The Hebb- Williams maze measures an animal's problem solving abilities by providing no spatial cues to find the target (Pritchett & Mulder, 2004).

Novel Object recognition. This is a simpler task that can be used to probe recognition memory. Two objects are presented to animal in an open field on trial 1, and these are explored. On trial 2, one object is replaced with a novel object and time spent interacting with the novel object is taken evidence of memory retention – I have seen one of these objects before, but not this one (Cohen and Stackman, 2015).

Contextual Fear conditioning is a hippocampal based learning task in which animals are placed in a novel environment and allowed to explore for several minutes before delivery of an aversive stimulus, typically a mild foot shock. Upon reintroduction to this same environment in the future (typically 24-48 hours after original training), animals will limit their exploration, the context of this chamber being associated with an aversive event. The degree of suppression of activity after training is taken as evidence of retention, i.e., memory (Curzon et al., 2009).

Trace fear conditioning. Standard fear conditioning paradigms require animals to make an association between a neutral conditioning stimulus (CS, a light or a tone) and an aversive stimulus (US, a footshock). The unconditioned response (CR) that is elicited upon delivery of the footshock US is freezing behavior. With repetition of CS/US delivery, the previously neutral stimulus comes to elicit the freezing response. This type of learning is dependent on the amygdala, a brain region associated with, but distinct from the hippocampus. Introducing a brief delay between presentation of the neutral CS and the aversive US, a trace period, requires the engagement of the amygdala and the hippocampus (Shors et al., 2001).

Operant Responding. Performance on operant responding reflects the cortex' ability to organize processes (Rabin et al., 2002).

In humans: A variety of standardized learning and memory tests have been developed for human neuropsychological testing, including children (Rohlman et al., 2008). These include episodic autobiographical memory, perceptual motor tests, short and long term memory tests, working memory tasks, word pair recognition memory; object location recognition memory. Some have been incorporated in general tests of intelligence (IQ) such as the Wechsler Adult Intelligence Scale (WAIS) and the Wechsler.

Modifications have been made and norms developed for incorporating of tests of learning and memory in children. Examples of some of these tests include:

Rey Osterieth Complex Figure test (RCFT) which probes a variety of functions including as visuospatial abilities, memory, attention, planning, and working memory (Shin et al., 2006).

Children's Auditory Verbal Learning Test (CAVLT) is a free recall of presented word lists that yields measures of Immediate Memory Span, Level of Learning, Immediate Recall, Delayed Recall, Recognition Accuracy, and Total Intrusions. (Lezak 1994; Talley, 1986).

Continuous Visual Memory Test (CVMT) measures visual learning and memory. It is a free recall of presented pictures/objects rather than words but that yields similar measures of Immediate Memory Span, Level of Learning, Immediate Recall, Delayed Recall, Recognition Accuracy, and Total Intrusions. (Lezak, 1984; 1994).

Story Recall from Wechsler Memory Scale (WMS) Logical Memory Test Battery, a standardized neurospychological test designed to measure memory functions (Lezak, 1994; Talley, 1986).

Autobiographical memory (AM) is the recollection of specific personal events in a multifaceted higher order cognitive process. It includes episodic memory- remembering of past events specific in time and place, in contrast to semantic autobiographical memory is the recollection of personal facts, traits, and general knowledge. Episodic AM is associated with greater activation of the hippocampus and a later and more gradual developmental trajectory. Absence of episodic memory in early life (infantile amnesia) is thought to reflect immature hippocampal function (Herold et al., 2015; Fivush, 2011).

Staged Autobiographical Memory Task. In this version of the AM test, children participate in a staged event involving a tour of the hospital, perform a series of tasks (counting footprints in the hall, identifying objects in wall display, buy lunch, watched a video). It is designed to contain unique event happenings, place, time, visual/sensory/perceptual details. Four to five months later, interviews are conducted using Children's Autobiographical Interview and scored according to standardized scheme (Willoughby et al., 2014).

Attentional set-shifting (ATSET) task. Measures the ability to relearn cues over various schedules of reinforcement (Heisler et al., 2015).

In Honey Bees: For over 50 years an assay for evaluating olfactory conditioning of the proboscis extension reflex (PER) has been used as a reliable method for evaluating appetitive learning and memory in honey bees (Guirfa and Sandoz, 2012; LaLone et al., 2017). These experiments pair a conditioned stimulus (e.g., an odor) with an unconditioned stimulus (e.g., sucrose) provided immediately afterward, which elicits the proboscis extension (Menzel, 2012). After conditioning, the odor alone will lead to the conditioned PER. This methodology has aided in the elucidation of five types of olfactory memory phases in honey bee, which include early short-term memory, late short-term memory, mid-term memory, early long-term memory, and late long-term memory (Guirfa and Sandoz, 2012). These phases are dependent on the type of conditioned stimulus, the intensity of the unconditioned stimulus, the number of conditioning trials, and the time between trials. Where formation of short-term memory occurs minutes after conditioning and decays within minutes, memory consolidation or stabilization of a memory trace after initial acquisition leads to

mid-term memory, which lasts 1 d and is characterized by activity of the cAMP-dependent PKA (Guirfa and Sandoz, 2012). Multiple conditioning trials increase the duration of the memory after learning and coincide with increased Ca2+-calmodulin-dependent PKC activity (Guirfa and Sandoz, 2012). Early long-term memory, where a conditioned response can be evoked days to weeks after conditioning requires translation of existing mRNA, whereas late long-term memory requires de novo gene transcription and can last for weeks (Guirfa andSandoz, 2012)."

### Regulatory Significance of the AO

A prime example of impairments in learning and memory as the adverse outcome for regulatory action is developmental lead exposure and IQ function in children (Bellinger, 2012). Most methods are well established in the published literature and many have been engaged to evaluate the effects of developmental thyroid disruption. The US EPA and OECD Developmental Neurotoxicity (DNT) Guidelines (OCSPP 870.6300 or OECD TG 426) as well as OECD TG 443 (OECD, 2018) both require testing of learning and memory (USEPA, 1998; OECD, 2007) advising to use the following tests passive avoidance, delayed-matching-to-position for the adult rat and for the infant rat, olfactory conditioning, Morris water maze, Biel or Cincinnati maze, radial arm maze, T-maze, and acquisition and retention of schedule-controlled behavior. These DNT Guidelines have been deemed valid to identify developmental neurotoxicity and adverse neurodevelopmental outcomes (Makris et al., 2009).

Also, in the frame of the OECD GD 43 (2008) on reproductive toxicity, learning and memory testing may have potential to be applied in the context of developmental neurotoxicity studies. However, many of the learning and memory tasks used in guideline studies may not readily detect subtle impairments in cognitive function associated with modest degrees of developmental thyroid disruption (Gilbert et al., 2012).

### References

Aggleton JP, Brown MW. (1999) Episodic memory, amnesia, and the hippocampal-anterior thalamic axis. Behav Brain Sci. 22: 425- 489.

Alexander RD (1990) Epigenetic rules and Darwinian algorithms: The adaptive study of learning and development. Ethology and Sociobiology 11:241-303.

Bellinger DC (2012) A strategy for comparing the contributions of environmental chemicals and other risk factors to neurodevelopment of children. Environ Health Perspect 120:501-507.

Burgess N (2002) The hippocampus, space, and viewpoints in episodic memory. Q J Exp Psychol A 55:1057-1080. Cohen, SJ and Stackman, RW. (2015). Assessing rodent hippocampal involvement in the novel object recognition task. A review. Behav. Brain Res. 285: 105-1176.

Cekanaviciute, E., S. Rosi and S. Costes. (2018), "Central Nervous System Responses to Simulated Galactic Cosmic Rays", International Journal of Molecular Sciences, Vol. 19/11, Multidisciplinary Digital Publishing Institute (MDPI) AG, Basel, https://doi.org/10.3390/ijms19113669.

Cohen, SJ and Stackman, RW. (2015). Assessing rodent hippocampal involvement in the novel object recognition task. A review. Behav. Brain Res. 285: 105-1176.

Curzon P, Rustay NR, Browman KE. Cued and Contextual Fear Conditioning for Rodents. In: Buccafusco JJ, editor. Methods of Behavior Analysis in Neuroscience. 2nd edition. Boca Raton (FL): CRC Press/Taylor & Francis; 2009.

D'Hooge R, De Deyn PP (2001) Applications of the Morris water maze in the study of learning and memory. Brain Res Brain Res Rev 36:60-90.

Doya K. (2000) Complementary roles of basal ganglia and cerebellum in learning and motor control. Curr Opin Neurobiol. 10: 732- 739.

Eichenbaum H (2000) A cortical-hippocampal system for declarative memory. Nat Rev Neurosci 1:41-50. Fivush R. The development of autobiographical memory. Annu Rev Psychol. 2011;62:559-82.

Gilbert ME, Sanchez-Huerta K, Wood C (2016) Mild Thyroid Hormone Insufficiency During Development Compromises Activity-Dependent Neuroplasticity in the Hippocampus of Adult Male Rats. Endocrinology 157:774-787.

Gilbert ME, Rovet J, Chen Z, Koibuchi N. (2012) Developmental thyroid hormone disruption: prevalence, environmental contaminants and neurodevelopmental consequences. Neurotoxicology 33: 842-52.

Gilbert ME, Sui L (2006) Dose-dependent reductions in spatial learning and synaptic function in the dentate gyrus of adult rats following developmental thyroid hormone insufficiency. Brain Res 1069:10-22.

Guirfa, M., Sandoz, J.C., 2012. Invertebrate learning and memory: fifty years of olfactory conditioning of the proboscis extension response in honeybees. Learn. Mem. 19 (2),

54–66.

Herold, C, Lässer, MM, Schmid, LA, Seidl, U, Kong, L, Fellhauer, I, Thomann,PA, Essig, M and Schröder, J. (2015). Neuropsychology, Autobiographical Memory, and Hippocampal Volume in “Younger” and “Older” Patients with Chronic Schizophrenia. Front. Psychiatry, 6: 53.

Hladik, D. and S. Tapio. (2016), "Effects of ionizing radiation on the mammalian brain", Mutation Research/Reviews in Mutation Research, Vol. 770, Elsevier B. b., Amsterdam, https://doi.org/10.1016/j.mrrev.2016.08.003.

Heisler, J. M. et al. (2015), "The Attentional Set Shifting Task: A Measure of Cognitive Flexibility in Mice", Journal of Visualized Experiments, 96, JoVe, Cambridge, https://doi.org/10.3791/51944.

LaLone, C.A., Villeneuve, D.L., Wu-Smart, J., Milsk, R.Y., Sappington, K., Garber, K.V., Housenger, J. and Ankley, G.T., 2017. Weight of evidence evaluation of a network of adverse outcome pathways linking activation of the nicotinic acetylcholine receptor in honey bees to colony death. STOTEN. 584-585, 751-775.

Lezak MD (1984) Neuropsychological assessment in behavioral toxicology--developing techniques and interpretative issues. Scand J Work Environ Health 10 Suppl 1:25-29.

Lezak MD (1994) Domains of behavior from a neuropsychological perspective: the whole story. Nebr Symp Motiv 41:23-55.

Makris SL, Raffaele K, Allen S, Bowers WJ, Hass U, Alleva E, Calamandrei G, Sheets L, Amcoff P, Delrue N, Crofton KM. (2009) A retrospective performance assessment of the developmental neurotoxicity study in support of OECD test guideline 426. Environ Health Perspect. Jan;117(1):17-25.

Menzel, R., 2012. The honeybee as a model for understanding the basis of cognition. Nat. Rev. Neurosci. 13 (11), 758–768.

Mitchell AS, Dalrymple-Alford JC, Christie MA. (2002) Spatial working memory and the brainstem cholinergic innervation to the anterior thalamus. J Neurosci. 22: 1922-1928.

OECD. 2007. OECD guidelines for the testing of chemicals/ section 4: Health effects. Test no. 426: Developmental neurotoxicity study. www.Oecd.Org/dataoecd/20/52/37622194.Pdf [accessed may 21, 2012].

OECD (2008) Nr 43 GUIDANCE DOCUMENT ON MAMMALIAN REPRODUCTIVE TOXICITY TESTING AND ASSESSMENT. ENV/JM/MONO(2008)16

Ono T. (2009) Learning and Memory. Encyclopedia of neuroscience. M D. Binder, N. Hirokawa and U. Windhorst (Eds). Springer- Verlag GmbH Berlin Heidelberg. pp 2129-2137.

Parihar, V. K. et al. (2020), "Sex-Specific Cognitive Deficits Following Space Radiation Exposure", Frontiers in Behavioral Neuroscience, Vol. 14, https://doi.org/10.3389/fnbeh.2020.535885.

Pritchett, K. and G. Mulder. (2004), "Hebb-Williams mazes.", Contemporary topics in laboratory animal science, Vol. 43/5, http://www.ncbi.nlm.nih.gov/pubmed/15461441.

Puig, M.V., Antzoulatos, E.G., Miller, E.K., 2014. Prefrontal dopamine in associative learning and memory. Neuroscience 282, 217– 229.

Rabin, B. M. et al. (2002), "Effects of Exposure to 56Fe Particles or Protons on Fixed-ratio Operant Responding in Rats", Journal of Radiation Research, Vol. 43/S, https://doi.org/10.1269/jrr.43.S225.

Roberts AC, Bill BR, Glanzman DL. (2013) Learning and memory in zebrafish larvae. Front Neural Circuits 7: 126.

Rohlman DS, Lucchini R, Anger WK, Bellinger DC, van Thriel C. (2008) Neurobehavioral testing in human risk assessment. Neurotoxicology. 29: 556-567.

Shin, MS, Park, SY, Park, SR, Oeol, SH and Kwon, JS. (2006). Clinical and empirical applications of the Rey-Osterieth complex figure test. Nature Protocols, 1: 892-899.

Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T, Gould E (2001) Neurogenesis in the adult is involved in the formation of trace

memories. Nature 410:372-376.

Stanton ME, Spear LP (1990) Workshop on the qualitative and quantitative comparability of human and animal developmental neurotoxicity, Work Group I report: comparability of measures of developmental neurotoxicity in humans and laboratory animals. Neurotoxicol Teratol 12:261-267.

Talley, JL. (1986). Memory in learning disabled children: Digit span and eh Rey Auditory verbal learning test. Archives of Clinical Neuropsychology, Elseiver.

Toscano CD, Guilarte TR. (2005) Lead neurotoxicity: From exposure to molecular effects. Brain Res Rev. 49: 529-554.

U.S.EPA. 1998. Health effects guidelines OPPTS 870.6300 developmental neurotoxicity study. EPA Document 712-C-98-239.Office of Prevention Pesticides and Toxic Substances.

Vorhees CV, Williams MT (2014) Assessing spatial learning and memory in rodents. ILAR J 55:310-332.

Willoughby KA, McAndrews MP, Rovet JF. Accuracy of episodic autobiographical memory in children with early thyroid hormone deficiency using a staged event. Dev Cogn Neurosci. 2014 Jul;9:1-11.

# Appendix 2

## List of Key Event Relationships in the AOP

### List of Adjacent Key Event Relationships

**Relationship: 919: Binding of agonist, Ionotropic glutamate receptors leads to Overactivation, NMDARs**

#### AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| Binding of agonists to ionotropic glutamate receptors in adult brain causes excitotoxicity that mediates neuronal cell death, contributing to learning and memory impairment. | adjacent | High | |
| Binding of chemicals to ionotropic glutamate receptors leads to impairment of learning and memory via loss of drebrin from dendritic spines of neurons | adjacent | High | Moderate |

#### Evidence Supporting Applicability of this Relationship

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| Human, rat, mouse | Human, rat, mouse | High | NCBI |
| human and other cells in culture | human and other cells in culture | High | NCBI |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| During brain development, adulthood and aging | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Unspecific | High |

Various studies suggest the existence of functional NMDA-like receptors in invertebrates (Xia et al., 2005). Fly and rodent NMDARs exhibit several important differences (Murphy and Glanzman, 1997). The expression and function of NMDA receptors in rodent and primates is well characterized in the existing literature.

#### Key Event Relationship Description

NMDARs can be activated indirectly through initial activation of KA/AMPARs as it happens in the case of DomA exposure. DomA is an agonist of presynaptic and postsynaptic KARs and sustained activation of these receptors by DomA results in massive ion flux and excessive release of glutamate from excitatory terminals causing depolarization of the postsynaptic neuron (as descibed in MIE). Upon this depolarization the Mg2+ block is removed from the pore of NMDARs, resulting in their activation allowing sodium, potassium, and, importantly, calcium ions to enter into a cell. The sustained exposure to DomA causes pathological overactivation of NMDARs. In the case of exposure to glufosinate NMDARs activation is triggered by direct, sustained binding of glufosinate to the NMDARs.

#### Evidence Supporting this KER

**Biological Plausibility**

NMDARs are unique among ligand-gated ion channels in that their activation requires binding of two co-agonists, glycine and endogenous neurotransmitter, L-glutamate. Physiologically, however, glycine and glutamate have distinct functions. While L-glutamate is released from specific presynaptic terminals, low concentrations of ambient glycine present at the synapse are thought to be sufficient to allow receptor activation. There is a clear understanding that binding of glutamate or its analogue will activate NMDA receptor (accepted dogma). The prolonged activation of NMDARs will lead to a pathological over-activation of a receptor leading to

excitotoxicity (minor role of KA/AMPARs), allowing high levels of calcium ions to enter the cell. However, KA/AMPARs play an important role for indirect NMDAR activation since (almost always) an initial activation of these receptors triggers depolarization of postsynaptic neurons that relieves the block of the channel pore by Mg2+, resulting in NMDAR activation. NMDA receptors are formed by a ligand binding domain (LBD) and an ion channel that are considered the core structural and functional elements of the receptors. There is a clear understanding of how agonist binding leads to channel opening that relies on structural (e.g. crystallography or NMR) and functional (e.g. UV and IR spectrometric measurements) experimental studies of the water-soluble LBD combined with functional studies of the intact receptor. After the initial agonist binding, a conformational change—so-called clam shell closure—that prevents agonist dissociation occurs followed by a conformational change in the ion channel that is tightly coupled to that in the LBD (reviewed in Traynelis et al., 2010). Consequently it can be stated that there is a clear structural and functional mechanistic understanding in this KER between MIE (Binding of agonist to glutamate ionotropic receptors) and KE1, NMDAR overactivation that, as explained above, can be triggered by direct binding to NMDAR or indirectly, through initial activation of KA/AMPARs as it happens in the case of exposure to glufosinate and DomA respectively, two stressors described in this AOP.

Indeed, domoic acid has a very strong affinity for the ionotropic glutamate receptors, the activation of which results in excitotoxicity, initiated by an integrative action of ionotropic receptors at both sides of the synapse blocking the channel from rapid desensitization. It has a synergistic effect with endogenous glutamate and it acts mainly as an agonist for presynaptic and postsynaptic kainate receptors. Activation of ionotropic receptors leads to the influx of Na+, K+ and Ca2+, particulary after activation of NMDARs. In combination with the inhibitory GABA neurotransmitter, glutamate contributes to the control of overall neuronal excitability.

Gufosinate (GLF) triggers alterations in glutamatergic signaling through direct binding and activation of NMDARs (Lantz et al., 2014: Matsumura et al., 2001). GLF agonist action at the NMDAR is expected to occur through interaction with the glutamate binding site and requires binding of the glycine co-agonist as well as release of the magnesium block from the channel pore. Additionally, the possible inhibition by GLF of the high affinity glutamate re-uptake transporter, especially GLT-I was studied to determine whether GLF could increase the levels of endogenous glutamate at the synaptic cleft, resulting in over activation of NMDARs. Such mechanism was excluded by Lantz (Lantz et al., 2014) but suggested by other studies (Watanabe and Sano, 1998).

**Empirical Evidence**

*Include consideration of temporal concordance here*

There is well established understanding of NMDAR activation by endogenous agonist glutamate that happens in the absence of the Mg2+ block under conditions of depolarized post-synaptic membrane(accepted dogma) (Blanke et al., 2009a and b; Enoki R, et al.,2004).

Single channel behavior of NMDARs from hippocampal CA1 neurons was studied using very low glutamate concentrations to improve temporal resolution of individual glutamate binding events. Openings resulting from individual receptor activations showed surprising complexity: they consist of a long cluster of bursts of openings. Furthermore, the NMDARs appeared to have different gating modes, occasionally entering periods of very high open probability (Gibb et al., 1991). Single channel analysis also provided insight in how NMDARs function at the synapse. In response to a brief pulse of glutamate, mimicking synaptic release, NMDARs activate slowly over hundreds of milliseconds and continue activating long after all glutamate has been removed from the synaptic cleft, thereby briefly “memorizing” the occurrence of a synaptic input. Single channel analysis of NR1 and NR2A receptors indicates that after a brief pulse of glutamate, receptors enter a high affinity closed state from which either channel opening or agonist unbinding occurs with approximately equal probability (Popescu et al., 2004). A single synaptic event is therefore expected to only partially activate NMDARs. Consequently, a closely spaced second pulse of agonist is able to further increase the open probability, endowing the NMDAR with an ability to decode synaptic input frequency.

**Domoic acid** is an agonist for presynaptic and postsynaptic kainate receptors, however indirectly also activates NMDA receptors. Kainate receptors are localized both at presynaptic and postsynaptic sites. At presynaptic sites, they directly affect transmitter release from both excitatory and inhibitory neuron terminals. At postsynaptic sites, kainate receptors lead to cell depolarization, which would bring the neuron closer to its spike firing threshold. By having this dual localization, kainate receptors help in the control of neuronal excitability. However, sustained activation of postsynaptic kainate receptors by domoic acid results in massive ion flux and excessive release of glutamate from excitatory terminals. The released glutamate in turn activates NMDA receptors, which have lost their physiologic Mg2++ block because of domoic acid–induced depolarization. The final event is an increase of NMDA-mediated Ca2++ flux and subsequent activation of intracellular pro-oxidative cascades and ion imbalances, eventually leading to excitotoxicity-mediated neuronal death (Babot et al., 2005;Giordano et al., 2006).

Kainate receptors are widely expressed in the hippocampus. Glutamatergic granule cells in the hippocampus express these receptors, suggesting that cell death found after domoic acid intoxication may be produced by hyperstimulation of NMDA receptor after glutamate is released in excess. In agreement with this hypothesis, the seriously damaged CA3 area of the hippocampus receives projections from hippocampal granule cells. Qiu and Curras-Collazo (Qiu et al., 2006a) elegantly demonstrated that domoic acid first targets kainate receptors in the hippocampus by blocking its effects in vivo with a kainate receptor antagonist. The sequential involvement of distinct glutamate receptors was confirmed and further elucidated in rat mixed cortical cell and hippocampal slice cultures (Jakobsen et al., 2002; Qiu et al., 2006b).

Using primary cultures of rodent cerebellar granule cells, an in vitro model mainly constituted by glutamatergic neurons that express both NMDA and kainate receptors it was proved that domoic acid increased glutamate release, intracellular calcium, and cell death, which were prevented by kainate and NMDA receptor antagonists (Berman and Murray, 1997; Vale-Gonzalez et al., 2006) confirming that DomA toxicity is mediated by both KA and NMDARs.

**Glufosinate** (GLF) and its primary metabolite N-acetylglufosinate (NAcGLF) interaction with NMDARs was studied in the primary culture of rat cortical neurons by performing [3H]CGP 39653 binding experiments. The results showed that their binding affinity to NMDAR (IC50, GLF 668 uM and NAcGLF approximately 100 uM) corresponded to the concentration that produce the highest increase of mean firing rate. Furthermore, they produced biphasic MFR profile, specific to NMDAR agonists. The obtained results suggest that GLF and NAcGLF can produce both effects,excitatory and inhibitory on network activity through direct activation of NMDARs (Lantz et al., 2014).

Direct activation of NMDARs by GLF is also suggested by in vivo studies where three NMDA receptor antagonists, dizocilpine, LY235959, and Compound 40, and AMPA/KA antagonist, NBQX, were co-administrated with glufosinate ammonium (80 mg/kg, intraperitoneally) in mice. Statistical analyses showed that the NMDA receptor antagonists markedly inhibited the GLF-induced convulsions, while the AMPA/KA receptor antagonist had no effect. These results suggest that the convulsion caused by glufosinate ammonium were mediated through activation of NMDA receptors (Matsumura et al., 2001).

**Uncertainties and Inconsistencies**

The increase in MFR induced by GLF in neuronal networks was significantly blocked by MK-801 but not entirely suggesting that GLF can increase activity in the MEA system through non-synaptic NMDARs, since these are not blocked by MK-801. It is not entirely clear whether GLF can work through an inhibition of the glutamate reuptake transporter, GLT-I, increasing the concentration of endogenous

glutamate at the synaptic cleft and subsequently resulting in over activation of NMDARs (Lantz et al., 2014: Watanabe and Sano, 1998). Further studies are necessary to determine whether this alternative mechanism of GLF-induced NMDAR overactivation takes place. Additionally GLF also modulates glutamine synthetase (GS) activity. Since, astrocytic GS in the brain participates in the metabolic regulation of glutamate (endogenous agonist of NMDAR) it is not clear if this pathway contributes to NMDAR activation too.

### References


Babot Z, Cristofol R, Sun˜ol C.,Excitotoxic death induced by released glutamate in depolarized primary cultures of mouse cerebellar granule cells is dependent on GABAA receptors and niflumic acidsensitive chloride channels. Eur J Neurosci., 2005, 21: 103–112.

Blanke ML, VanDongen AMJ., Activation Mechanisms of the NMDA Receptor. In: Van Dongen AM, editor. Biology of the NMDA Receptor. Boca Raton (FL): CRC Press; 2009a. Chapter 13. Frontiers in Neuroscience.

Blanke ML., and Antonius M.J. VanDongen, Activation Mechanisms of the NMDA Receptor in Biology of the NMDA Receptor,2009b, Chapter 13, Van Dongen AM, editor. Boca Raton (FL): CRC Press.

Berman FW, Murray TF., Domoic acid neurotoxicity in cultured cerebellar granule neurons is mediated predominantly by NMDA receptors that are activated as a consequence of excitatory amino acid release. J Neurochem., 1997, 69: 693–703.

Enoki R, et al., NMDAR-mediated depolarizing after-potentials in the basal dendrites of CA1 pyramidal neurons. Neurosci Res., 2004, 48: 325-337.

Gibb AJ, Colquhoun D. Glutamate activation of a single NMDAR-channel produces a cluster of channel openings. Proc. R. Soc. Lond. (Biol.) 1991, 243: 39-47.

Giordano G, White CC, McConnachie LA, Fernandez C, Kavanagh TJ, Costa LG., Neurotoxicity of domoic Acid in cerebellar granule neurons in a genetic model of glutathione deficiency. Mol Pharmacol., 2006, 70: 2116–2126.

Jakobsen B, Tasker A, Zimmer J., Domoic acid neurotoxicity in hippocampal slice cultures. Amino Acids, 2002, 23: 37–44.

Lantz Stephen R , Cina M. Mack , Kathleen Wallace, Ellen F. Key , Timothy J. Shafer , John E. Casida, Glufosinate binds to N-methyl-D-aspartate receptors and increases neuronal network activity in vitro. NeuroToxicology, 2014, 45: 38–47.

Matsumura N1, Takeuchi C, Hishikawa K, Fujii T, Nakaki T., Glufosinate ammonium induces convulsion through N-methyl-D-aspartate receptors in mice. Neurosci Lett., 2001, 304(1-2): 123-5.

Murphy GG, Glanzman DL., Mediation of classical conditioning in Aplysia californica by long-term potentiation of sensorimotor synapses. Science, 1997, 278: 467-78.

Popescu G, et al. Reaction mechanism determines NMDAR response to repetitive stimulation. Nature. 2004, 430: 790-799.

Qiu S, Curras-Collazo MC., Histopathological and molecular changes produced by hippocampal microinjection of domoic acid. Neurotoxicol Teratol., 2006a, 28: 354–362.

Qiu S, Pak CW, Curras-Collazo MC., Sequential involvement of distinct glutamate receptors in domoic acid-induced neurotoxicity in rat mixed cortical cultures: Effect of multiple dose/duration paradigms, chronological age, and repeated exposure. Toxicol Sci., 2006b, 89: 243–256.

Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, Hansen KB, Yuan H, Myers SJ, Dingledine R., Glutamate receptor ion channels: structure, regulation, and function. Pharmacol Rev., 2010, 62(3):405-96.

Vale-Gonzalez C, Alfonso A, Sun˜ol C, Vieytes MR, Botana LM., Role of the plasma membrane calcium adenosine triphosphatase on domoate-induced intracellular acidification in primary cultures of cerebellar granule cells. J Neurosci Res., 2006, 84: 326–337.

Watanabe T1, Sano T., Neurological effects of glufosinate poisoning with a brief review. Hum Exp Toxicol. 1998, 17: 35-9.

Xia S, et al., NMDARs mediate olfactory learning and memory in Drosophila. Curr Biol., 2005, 15:603-618.


## Relationship: 361: Overactivation, NMDARs leads to Increased, Intracellular Calcium overload

### AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| Binding of agonists to ionotropic glutamate receptors in adult brain causes excitotoxicity that mediates neuronal cell death, contributing to learning and memory impairment. | adjacent | Moderate | |
| Acetylcholinesterase Inhibition Leading to Neurodegeneration | adjacent | High | Moderate |
| Calcium overload in dopaminergic neurons of the substantia nigra leading to parkinsonian motor deficits | adjacent | Not Specified | Not Specified |
| Binding of chemicals to ionotropic glutamate receptors leads to impairment of learning and memory via loss of drebrin from dendritic spines of neurons | adjacent | High | High |

### Evidence Supporting Applicability of this Relationship

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| zebrafish | Danio rerio | Low | NCBI |

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| rat | Rattus norvegicus | High | NCBI |
| mouse | Mus musculus | High | NCBI |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| All life stages | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Unspecific | High |

NMDARs have been shown to regulate calcium ion flow in a variety of species including zebrafish and rats (Horzmann and Freeman, 2016, el Nasr et al., 1990).

### Key Event Relationship Description

The NMDA receptor is distinct from the other glutamate receptors in two ways: first, it is both ligand-gated and voltage-dependent; second, it requires co-activation by two ligands: glutamate and either glycine or D-serine. Following membrane depolarization, the co-agonists, L-glutamate and glycine must bind to their respective sites on the receptor to open the channel. On activation, the NMDA receptor allows the influx of extracellular calcium ions into the postsynaptic neuron and neurotransmission occurs (reviewed in Higley and Sabatini, 2012). Calcium flux through NMDA receptors is also thought to be critical in synaptic plasticity, a cellular mechanism for learning and memory. Indeed, NMDA receptor–dependent synaptic potentiation (LTP) and depression (LTD) are two forms of activity-dependent long-term changes in synaptic efficacy that are believed to represent cellular correlates of learning and memory processes. The best characterized form of NMDA receptor-dependent LTP and LTD occurs between CA3 and CA1 pyramidal neurons of the hippocampus (Luscher and Malenka, 2012). It is now well established that modest activation of NMDARs leads to modest increases in postsynaptic calcium, triggering LTD, whereas much stronger activation of NMDARs leading to much larger increases in postsynaptic calcium are required to trigger LTP (Luscher and Malenka, 2012). The high-frequency stimulation causes a strong temporal summation of the excitatory postsynaptic potentials, and depolarization of the postsynaptic cell is sufficient to relieve the Mg2+ block of the NMDAR and allow a large amount of calcium to enter into the post-synaptic cells.

### Evidence Supporting this KER

#### Biological Plausibility

There is structural and functional mechanistic understanding supporting this relationship between NMDAR overactivation and increased intracellular calcium.

The relationship between the upstream and downstream key event is plausible as the expression of the functional NMDA receptors is commonly carried out or assessed by Ca2+ imaging method. Calcium imaging techniques have been extensively utilized in the literature to investigate the potential interactions between NMDA-evoked Ca2+ influx and NMDA receptor activation. Approximately 15% of the current through NMDA receptors is mediated by Ca2+ under physiological conditions (Higley and Sabatini, 2012).

It has been shown that less than five and, occasionally, only a single NMDA receptor opens under physiological conditions, causing a total Ca2+ influx of about 6000 ions into a spine head reaching a concentration of ~10 µm (Higley and Sabatini, 2012). However, the majority of the ions are rapidly eliminated by binding to Ca2+ proteins, reaching ~1 µM of free Ca2+ concentration (Higley and Sabatini, 2012).

It has been shown that in rat primary forebrain cultures the intracellular Ca2+ increases after activation of the NMDA receptor through administration of NMDA but this increase in Ca2+ is blocked when the cells are cultured under Ca2+ free conditions, demonstrating that the NMDA-evoked increase in intracellular Ca2+ derives from extracellular and not intracellular sources (Liu et al., 2013).

Indirect mechanism of domoic acid (DA) induced overactivation of NMDARs that result in Ca2+ overload: depolarization of the pre-synaptic cell activates the release of endogenous Ca2+ which mobilizes vesicles containing GLU to the membrane surface. Glutamate (GLU) is then released into the synaptic cleft by exocytosis where it is able to interact with cell surface receptors. Exogenous DA can interact within the synaptic cleft with each of the three ionotropic receptor subtypes including the kainate, AMPA, and NMDA receptors on cell membranes. Activation of the kainate and AMPA receptors results in release of Ca2+ via coupled ion channels, into the post-synaptic cell. DA is also able to bind to NMDA receptors that are linked to both Ca2+ and NA/K+ ion channels and results in a cellular influx of both Na+ and Ca2+. Unlike GLU, DA induces prolonged receptor activation causing a constant influx of cations into the cell and the appropriate chemical cues for desensitization are blocked. The excess intracellular Ca2+ causes disruption of cellular function, cell swelling and ultimately cell death (Lefebvre and Robertson,2010).

Glufosinate (GLF) is the methylphosphinate analog of glutamate that directly can activate NMDARs (Lantz et al., 2014, Matsumura et al., 2001, Faro et al., 2013) (as described in KE: *NMDARs, Binding of agonist*). It is well established in the existing literature that activation of NMDARs leads to the intra-cellular Ca2+ overload and based on this assumption it can be suggested that an exposure to GLF leads to increased intra-cellular calcium levels.

#### Empirical Evidence

*Include consideration of temporal concordance here*

**Domoic acid (DomA)**

- Treatment of mouse cerebellar granule neurons (CGNs) with 1 or 10 µM DomA causes increase of intracellular Ca2+ by approximately 5 or 8 fold compared to controls, respectively (Giordano et al., 2006). Interestingly, when the cells are exposed simultaneously to DomA and the NMDA receptor antagonist MK-801, the Ca2+ levels measured are close to control levels, indicating that the Ca2+ elevation evoked by DomA involves activation of NMDA receptors (Giordano et al., 2006).
- The same research group has performed a time course study by applying a high and a low DomA concentration and using CGNs from Gclm (+/+) and Gclm (−/−) mice lacking glutathione (Giordano et al., 2007). The low DomA dose (0.1µM) causes a small and

delayed increase in intracellular Ca2+ concentration with a full recovery by 20 min. When the experiment is performed in the absence of extracellular calcium, this increase of intracellular Ca2+ levels in the presence of DomA is abolished, indicating that this change in homeostasis of Ca2+ is due to ion entry from outside the cell. However, this recording of intracellular Ca2+ is antagonised only by NBQX (AMPA receptor antagonist), but not by MK-801 (NMDA receptor antagonist). On the other hand, the higher DomA concentration (10µM) causes a rapid and robust increase in intracellular Ca2+, which lasts even after 25 min. This effect is antagonized by both NBQX and MK-801, suggesting that not only AMPA but also NMDA receptors are involved in Ca2+ elevation evoked by DomA at high doses (Giordano et al., 2007).

- In an earlier study, the time course and concentration dependence of the increase in intracellular Ca2+ stimulated by DomA has been examined in 10-13 day-in-culture CGNs (Berman et al., 2002). DomA produces a rapid and concentration-dependent increase in intracellular Ca2+, showing the maximal increase at 10 µM DomA (Berman et al., 2002). At this concentration, fluo-3 fluorescence that is used to measure Ca2+ elevates rapidly during the first 40 s of exposure, increases more slowly before peaking at 3.5 min, after which the signal diminishes steadily over the 30 min course of the experiment to 55% of peak values. The EC50 for DomA-induced increase in intracellular Ca2+ is 0.61 µM. In the same study, the NMDA receptor antagonist MK-801 significantly reduced both peak and final plateau of intracellular Ca2+ by 30 and 70%, respectively (Berman et al., 2002).
- These three studies (Giordano et al., 2006; 2007; Berman et al., 2002) do not provide a simultaneous measurement of NMDA receptor activation by DomA and intracellular Ca2+ levels. However, they do provide indirect evidence of NMDA receptor activation involvement in increased intracellular Ca2+ concentrations induced by DomA as they have used known antagonists of the NMDA receptors that reverses the situation in both KEs (blocking upstream KE will block downstream KE).
- In an in vivo study it was indirectly shown that the microinjection to adult male Sprague Dawley rats of 10 µM DomA increased intracellular Ca2+ levels. A significant upregulation of phosphorylated calcium-calmodulin-dependent kinase II (CaMKII) and phosphorylated cAMP response element binding protein (CREB) levels was recorded, possibly due to increased intracellular Ca2+ levels induced by DomA (Qiu and Currás-Collazo, 2006).

In CGNs, the co-treatment with 10 µM DomA and the kainate/AMPA receptor antagonist NBQX maintains Ca2+ levels near to control levels, suggesting that the Ca2+ elevation evoked by DomA is mediated by the activation of both AMPA/kainate and of NMDA receptors (Giordano et al., 2006).

The voltage-sensitive Ca2+ channel (VSCC) blocker nifedipine (5 µM) and NBQX (10 µM), a competitive AMPA/kainate receptor antagonist reduces the peak and final intracellular Ca2+ concentration in CGNs (Berman et al., 2002), strengthening the view that the increase of Ca2+ influx is not only mediated by NMDA receptors but also by AMPA/kainate receptors and VSCCs.

Table 1: Summary of available data describing responses of intracellular calcium to NMDA receptor activation. DA, DomA = Domoic Acid. Glu = Glutamate. NMDA = N-methyl-D-aspartate. The following are NMDA receptor (NMDAR) antagonists: D-AP5 = D-2-amino-5-phosphonopentanoate. MK-801 = Dizocilpine.

| Stressor | Experimental Model | Tested concentrations | Exposure route | Exposure duration | Overactivation of NMDAR (KE up) (measurements, quantitative if available) | Increased intracelllular Ca 2+ levels (KE down) (measurements, quantitative if available) | References | Temporal Relationship | Dose-response relationship | Incidence | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DomA | Mouse cerebellar granule neurons (CGNs) from Gclm (+/+) and Gclm (−/−) mice | 0.01 to 10 µM | | Time course (15 to 120 min) | | 5 and 8 fold increase of [Ca2+]i compared to controls. | Giordano et al., 2006 | | | | The cells were exposed simultaneously to DA and the NMDA receptor antagonist MK-801 and the Ca2+ levels were found to be close to control levels, indicating that the Ca2+ elevation evoked by DA involves activation of NMDA receptors. |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DomA | CGNs from Gclm (+/+) and Gclm (−/−) mice | 0.01 to 10 µM | | Time course (0 to 25 min) | | 0.1µM domoic acid caused a small and delayed increase (4 fold) in [Ca2+]i, with a full recovery by 20 min.In contrast, the higher concentration of domoic acid (10µM) caused a rapid and robust increase (8 fold) in [Ca2+]i, which was still elevated after 25 min.<br>0.1µM DA increases [Ca2+]M by about 3 fold, with a delay of about 15 min. In contrast, no changes in [Ca2+]M were observed following 10µM of DA. | Giordano et al., 2007 | | | | At the low concentration (0.1µM), the recording of intracellular Ca2+ was antagonized only by NBQX (AMPA receptor antagonist), but not by MK-801 (NMDA receptor antagonist). On the other hand, the higher DA concentration (10µM) caused a rapid and robust increase in intracellular Ca2+ . This effect was antagonized by both NBQX and MK-801, suggesting the importance of NMDA receptors in Ca2+ elevation evoked by DA but only at high doses |
| DomA | 10-13 DIV CGNs obtained from 8-day-old Sprague–Dawley rats | 0.1 to 30 µM | | Time course (0 to 45 min) | | EC50 for DA-induced increase in intracellular Ca2+ was 0.61 µM | Berman et al., 2002 | | | | The NMDA receptor antagonist MK-801 significantly reduced both peak and final plateau of intracellular Ca2+ by 30 and 70%, respectively |
| DomA | Adult male Sprague Dawley rats | 10 µM | Brain microinjection | | | Increased phosphorylated CaMKII and phosphorylated CREB levels | Qiu and Currás-Collazo, 2006 | | | | |
| Glutamate, NMDA | Mouse cortical astrocytes | Glutamate: 100 µM<br>NMDA: 20 µM | | Brief (1s application) | | Increased intracellular Ca2+ measured through Fluo-3 Flourescence | Palygin et al., 2011 | | | | Provides time-series data of intracellular calcium measured through fluoresence given an application of Glu, NMDA, or Glu + D-AP5 in mouse cortical astrocytes. Cells were additionally exposed to D-AP5, an NMDA antagonist, and showed reduced fluorscence changes. (added by DS for AOP 281) |
| Glutamate | Cultured rat hippocampal neurons | 500 µM | | Time course (0 to 45 minutes) | | Increased intracellular Ca2+ measured through Fura-2 Flourescence | Michaels and Rothman, 1990 | | | | Provides time-series data of intracellular calcium measured through fluoresence, as well as directly providing calculated intracellular calcium concentrations in response to high concentrations of applied Glu, both alone and with antagonists. (added by DS for AOP 281) |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| NMDA, Glutamate | Neocortical neurons of Swiss-Webster mice | Glutamate: 300 µM<br>NMDA: 300 µM | | Time course (0 to 20 minutes) and (0 to 2 minutes) | | Increased intracellular Ca2+ measured through Fura-2/AM, Fura-2/K+, Fura-2/dextran, BTC | Hyrc et al., 1997 | | | | Provides time-series data of intracellular calcium measured through a variety of fluorsence calcium indicators given an application of the selective agonist NMDA. (added by DS for AOP 281) |
| Glutamate | Computational model (CA1 pyramidal neuron) | | | | | Models the concentration of Ca2+ in spine(s) of neuron | Hu et al., 2018 | | | | Developed a computational model of a glutamatergic spine which models intracellular calcium dynamics and sources of calcium influx including activation of NMDA receptors. (added by DS for AOP 281) |

**Glufosinate (GLF)**

There are no data showing that an exposure to GLF causes an increase in intra-cellular calcium. Such assumption can be proposed based on a fact that GLF directly activates NMDR as described in the MIE and other relevant KEs of this AOP.

**Uncertainties and Inconsistencies**

A case of a 59-yr-old woman who ingested a herbicide containing glufosinate was suffering from severe intoxication, however, she did not develop convulsions, which experimentally occurs in rats treated with GLF (Koyama et al., 1994) and is described in other human cases (Watanabe and Sano 1998).

## References


Berman FW, LePage KT, Murray TF., Domoic acid neurotoxicity in cultured cerebellar granule neurons is controlled preferentially by the NMDA receptor Ca(2+) influx pathway. Brain Res., 2002, 924: 20-29.

el Nasr, M. S., Peruche, B., Roβberg, C., Mennel, H.-D. & Krieglstein, J. 1990. Neuroprotective effect of memantine demonstrated in vivo and in vitro. *European Journal of Pharmacology,* 185**,** 19-24. DOI: https://doi.org/10.1016/0014-2999(90)90206-L.

Faro LR, Ferreira Nunes BV, Alfonso M, Ferreira VM, Durán R., Role of glutamate receptors and nitric oxide on the effects of glufosinate ammonium, an organophosphate pesticide, on in vivo dopamine release in rat striatum. Toxicology., 2013, Sep 15, 311: 154-61.

Giordano G, White CC, McConnachie LA, Fernandez C, Kavanagh TJ, Costa LG., Neurotoxicity of domoic Acid in cerebellar granule neurons in a genetic model of glutathione deficiency. Mol Pharmacol. 2006., 70: 2116-2126.

Giordano G, White CC, Mohar I, Kavanagh TJ, Costa LG., Glutathione levels modulate domoic acid-induced apoptosis in mouse cerebellar granule cells. Toxicol Sci., 2007, 100: 433-444.

Higley MJ, Sabatini BL., Calcium signalling in dendritic spines. Cold Spring Harb Perspect Biol., 2012, 4: a005686.

Horzmann, K. A. & Freeman, J. L. 2016. Zebrafish get connected: investigating neurotransmission targets and alterations in chemical toxicity. *Toxics,* 4.

Hu, E., Mergenthal, A., Bingham, C. S., Song, D., Bouteiller, J. M. & Berger, T. W. 2018. A Glutamatergic Spine Model to Enable Multi-Scale Modeling of Nonlinear Calcium Dynamics. *Front Comput Neurosci,* 12**,** 58. DOI: 10.3389/fncom.2018.00058.

Hyrc, K., Handran, S. D., Rothman, S. M. & Goldberg, M. P. 1997. Ionized intracellular calcium concentration predicts excitotoxic neuronal death: observations with low-affinity fluorescent calcium indicators. *J Neurosci,* 17**,** 6669-77. DOI: 10.1523/jneurosci.17-17-06669.1997.

Koyama K, Andou Y, Saruki K, Matsuo H., Delayed and severe toxicities of a herbicide containing glufosinate and a surfactant. Vet Hum Toxicol., 1994, 36: 17-8.

Lantz Stephen R , Cina M. Mack , Kathleen Wallace, Ellen F. Key , Timothy J. Shafer , John E. Casida., Glufosinate binds N-methyl-D aspartate receptors and increases neuronal network activity in vitro. NeuroToxicology, 2014, 45: 38–47.

Lefebvre KA, Robertson A. Domoic acid and human exposure risks: a review. Toxicon. 2010 Aug 15;56(2):218-30.

Liu F, Patterson TA, Sadovova N, Zhang X, Liu S, Zou X, Hanig JP, Paule MG, Slikker W Jr, Wang C., Ketamine-induced neuronal damage and altered N-methyl-D-aspartate receptor function in rat primary forebrain culture. Toxicol Sci., 2013, 131: 548-557.

Luscher C. and Robert C. Malenka. NMDA Receptor-Dependent Long-Term Potentiation and Long-Term Depression (LTP/LTD). Cold Spring Harb Perspect Biol., 2012;4:a005710.

Matsumura N, Takeuchi C, Hishikawa K, Fujii T, Nakaki T., Glufosinate ammonium induces convulsion through N-methyl-D-aspartate receptors in mice. Neurosci Lett., 2001, 304(1-2): 123-5.

Michaels, R. L. and Rothman, S. M. 1990. Glutamate neurotoxicity in vitro: antagonist pharmacology and intracellular calcium concentrations. *J Neurosci,* 10**,** 283-92. DOI: 10.1523/jneurosci.10-01-00283.1990.

Palygin, O., Lalo, U. & Pankratov, Y. 2011. Distinct pharmacological and functional properties of NMDA receptors in mouse cortical astrocytes. *British Journal of Pharmacology,* 163**,** 1755-1766. DOI: 10.1111/j.1476-5381.2011.01374.x.

Qiu S, Currás-Collazo MC., Histopathological and molecular changes produced by hippocampal microinjection of domoic acid. Neurotoxicol Teratol., 2006, 28: 354-362.

Watanabe T, Sano T., Neurological effects of glufosinate poisoning with a brief review. Hum Exp Toxicol. 1998, 17: 35-9.

## Relationship: 3091: Increased, Intracellular Calcium overload leads to Loss of drebrin

### AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| Binding of chemicals to ionotropic glutamate receptors leads to impairment of learning and memory via loss of drebrin from dendritic spines of neurons | adjacent | High | Moderate |

### Evidence Supporting Applicability of this Relationship

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| human | Homo sapiens | | NCBI |
| rat | Rattus norvegicus | High | NCBI |
| mouse | Mus musculus | High | NCBI |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| Fetal | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Male | High |
| Female | High |

### Key Event Relationship Description

Calcium modulates drebrin protein stability in neuronal cultures in ways that depend on both concentration and stimulus. NMDA receptor overactivation elevates intracellular calcium leads to loss of drebrin from dendritic spines of cultured coltcal neurons, hippocampal neurons and in vivo animal study.

Glutamate stimulation induced disappearance of drebrin immunostaining from dendritic spines but led to appearance of drebrin immunostaining in dendritic shafts and somata. The glutamate-induced shift of drebrin immunostaining was blocked by an NMDA receptor antagonist (Sekino, et al. 2006). Loss of drebrin from dendritic spines induced by hight-potasium stimulation or glutamate aplication was inhibited by EGTA and AP5 (Mizui, et al. 2014). Long term potentiation stimulation induces $Ca^{2+}$ entry through N-methyl-d-aspartate (NMDA) receptors, which causes drebrin exodus from dendritic spines (Koganezawa , et al 2017). Under NMDA-induced excitotoxic conditions with 1 mM $CaCl_2$, Chimura et al. (2015) report that drebrin A undergoes calpain-dependent degradation, with Western blotting and immunocytochemistry revealing the emergence of approximately 40 kDa fragments—a change prevented by calpain inhibitor-I.

### Quantitative Understanding of the Linkage

Protein quantification methods: Western blotting using specific antibodies (C-term and DAS2).

Under NMDA-induced excitotoxic conditions with 1 mM $CaCl_2$, Chimura et al. (2015) report that drebrin A undergoes calpain-dependent degradation, with Western blotting and immunocytochemistry revealing the emergence of approximately 40 kDa fragments—a change prevented by calpain inhibitor-I.

**Known modulating factors**

| Modulating Factor (MF) | MF Specification | Effect(s) on the KER | Reference(s) |
|---|---|---|---|
| calpain | high | increase | Chimura, et al. 2015 |

### References

Appearance order

Sekino Y, Tanaka S, Hanamura K, Yamazaki H, Sasagawa Y, Xue Y, Hayashi K, Shirao T. Activation of N-methyl-D-aspartate receptor induces a shift of drebrin distribution: disappearance from dendritic spines and appearance in dendritic shafts. Mol Cell Neurosci. 2006 Mar;31(3):493-504. doi: 10.1016/j.mcn.2005.11.003. Epub 2005 Dec 20. PMID: 16368245.

Mizui T, Sekino Y, Yamazaki H, Ishizuka Y, Takahashi H, Kojima N, Kojima M, Shirao T. Myosin II ATPase activity mediates the long-term potentiation-induced exodus of stable F-actin bound by drebrin A from dendritic spines. PLoS One. 2014 Jan 22;9(1):e85367. doi: 10.1371/journal.pone.0085367. PMID: 24465547; PMCID: PMC3899004.

Koganezawa N, Hanamura K, Sekino Y, Shirao T. The role of drebrin in dendritic spines. Mol Cell Neurosci. 2017 Oct;84:85-92. doi: 10.1016/j.mcn.2017.01.004. Epub 2017 Feb 1. PMID: 28161364.

Chimura T, Launey T, Yoshida N. Calpain-Mediated Degradation of Drebrin by Excitotoxicity In vitro and In vivo. PLoS One. 2015 Apr 23;10(4):e0125119. doi: 10.1371/journal.pone.0125119. PMID: 25905636; PMCID: PMC4408054.

## Relationship: 3298: Loss of drebrin leads to Dendritic spine abnormality

### AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| Binding of chemicals to ionotropic glutamate receptors leads to impairment of learning and memory via loss of drebrin from dendritic spines of neurons | adjacent | High | High |

### Evidence Supporting Applicability of this Relationship

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| human, mouse, rat | human, mouse, rat | High | NCBI |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| During development and at adulthood | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Mixed | Not Specified |

rat

mouse

### Key Event Relationship Description

Drebrin interacts with actin filaments to stabilize and reorganize the actin cytoskeleton within dendritic spines, and plays important roles in regulating spine morphology and synaptic plasticity. Loss of drebrin results in a reduction of mature mushroom-shaped spines and an increase in thin or immature filopodia-like spines.

### Evidence Supporting this KER

Early growth response-1 (Egr-1), an inducible zinc finger transcription factor, down-regulates drebrin expression. Golgi staining analyses revealed reduced drebrin protein and dendritic spine density as well as reduced expression of synaptic markers in in vitro hippocampal neurons over-expressing Egr-1 and in vivo indu cible mouse model of Egr-1. (Cho et al. 2017)
Suppression of the upregulation of drebrin adult isoform (drebrin A) by antisense oligonucleotides (AOD) against it attenuated synaptic clustering of PSD-95, as well as clustering of drebrin and F-actin. The application of AOD significantly decreased the density of cluster-type filopodia at 14 DIV(Takahashi et al. 2003)

Drebrin depletion also altered dendritic spine formation, morphology, and reduced levels of dopamine receptor D1 in dendritic spines as evaluated using immunohistochemistry/confocal microscopy. (Jung et al.2015)

**Biological Plausibility**

high

**Empirical Evidence**

high

### Quantitative Understanding of the Linkage

Quantitative understanding of the abnormality of spine morpjology is still uncertain.

**Time-scale**

During development (Takahashi et al. 2003; Jung et al. 2015)
Drebrin loss from dendritic spines of cultured neurons induced by glutamate stimulation alters dendritic spine morphology 5 min after the stimulation (Mizui et al. 2014)

### References

Cho C, MacDonald R, Shang J, Cho MJ, Chalifour LE, Paudel HK. Early growth response-1-mediated down-regulation of drebrin correlates with loss of dendritic spines. J Neurochem. 2017 Jul;142(1):56-73. doi: 10.1111/jnc.14031. Epub 2017 Apr 26. PMID: 28369888.

Takahashi H, Sekino Y, Tanaka S, Mizui T, Kishi S, Shirao T. Drebrin-dependent actin clustering in dendritic filopodia governs synaptic targeting of postsynaptic density-95 and dendritic spine morphogenesis. J Neurosci. 2003 Jul 23;23(16):6586-95. doi: 10.1523/JNEUROSCI.23-16-06586.2003. PMID: 12878700; PMCID: PMC6740629.

Jung G, Kim EJ, Cicvaric A, Sase S, Gröger M, Höger H, Sialana FJ, Berger J, Monje FJ, Lubec G. Drebrin depletion alters neurotransmitter receptor levels in protein complexes, dendritic spine morphogenesis and memory-related synaptic plasticity in the mouse hippocampus. J Neurochem. 2015 Jul;134(2):327-39. doi: 10.1111/jnc.13119. Epub 2015 Apr 29. PMID: 25865831.

## Relationship: 3301: Dendritic spine abnormality leads to Dysfunctional synapses

### AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| Binding of chemicals to ionotropic glutamate receptors leads to impairment of learning and memory via loss of drebrin from dendritic spines of neurons | adjacent | High | High |

### Evidence Supporting Applicability of this Relationship

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| human, mouse, rat | human, mouse, rat | High | NCBI |
| human | Homo sapiens | High | NCBI |
| rat | Rattus norvegicus | High | NCBI |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| During brain development, adulthood and aging | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Mixed | Not Specified |

### Key Event Relationship Description

Activity-dependent plasticity of synaptic structure and function is closely regulated by F-actin dynamics within dendritic spines (Matus, 1999, 2000). Dendritic spines are small, actin-rich protrusions extending from neuronal dendrites. They constitute the postsynaptic compartment of most excitatory synapses and serve as major sites for information processing and storage in the brain. Structural changes in dendritic spine morphology, such as alterations in spine shape and size, correlate strongly with the strength of excitatory synaptic connections. These morphological modifications critically depend on remodeling processes within the underlying actin cytoskeleton (Hotulainen et al., 2010). The formation, maintenance, and modulation of dendritic spines are primarily governed by the dynamics of filamentous actin (F-actin), which is tightly regulated through interactions with various actin-binding proteins (ABPs) and postsynaptic signaling molecules (Borovac et al., 2018). Consequently, abnormalities in dendritic spine structure arising from disrupted actin dynamics can lead directly to synaptic dysfunction.

### Evidence Supporting this KER

Chemicals that disrupt the dynamics of the actin cytoskeleton affect synaptic plasticity in a dose-dependent manner.

Cytochalasin D, cytochalasin B, and latrunculin A all impaired the maintenance of LTP induced by brief high-frequency stimulation. (Krucker T et al. 2000)

Latrunculin A:  Inhibition of actin polymerization with latrunculin A impaired late phase of LTP without affecting the initial amplitude and early maintenance of LTP. These observations suggest that mechanisms regulating the spine actin cytoskeleton contribute to the persistence of LTP. (Fukazawa et al. 2003)

**Biological Plausibility**

high

### Quantitative Understanding of the Linkage

**Time-scale**

minites

### References

Borovac J, Bosch M, Okamoto K. Regulation of actin dynamics during structural plasticity of dendritic spines: Signaling messengers and actin-binding proteins. Mol Cell Neurosci. 2018 Sep;91:122-130. doi: 10.1016/j.mcn.2018.07.001. Epub 2018 Jul 9. PMID: 30004015.

Matus A. Postsynaptic actin and neuronal plasticity. Curr Opin Neurobiol. 1999 Oct;9(5):561-5. doi: 10.1016/S0959-4388(99)00018-5. PMID: 10508747.

Matus A. Actin-based plasticity in dendritic spines. Science. 2000 Oct 27;290(5492):754-8. doi: 10.1126/science.290.5492.754. PMID: 11052932.

Hotulainen P, Hoogenraad CC. Actin in dendritic spines: connecting dynamics to function. J Cell Biol. 2010 May 17;189(4):619-29. doi: 10.1083/jcb.201003008. Epub 2010 May 10. PMID: 20457765; PMCID: PMC2872912.

Krucker T, Siggins GR, Halpain S. Dynamic actin filaments are required for stable long-term potentiation (LTP) in area CA1 of the hippocampus. Proc Natl Acad Sci U S A. 2000 Jun 6;97(12):6856-61. doi: 10.1073/pnas.100139797. PMID: 10823894; PMCID: PMC18765.

Fukazawa Y, Saitoh Y, Ozawa F, Ohta Y, Mizuno K, Inokuchi K. Hippocampal LTP is accompanied by enhanced F-actin content within the dendritic spine that is essential for late LTP maintenance in vivo. Neuron. 2003 May 8;38(3):447-60. doi: 10.1016/s0896-6273(03)00206-x. PMID: 12741991.

## Relationship: 2504: Dysfunctional synapses leads to Neuronal network function, Decreased

### AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| A cholesterol/glucose dysmetabolism initiated Tau-driven AOP toward memory loss (AO) in sporadic Alzheimer's Disease with plausible MIE's plug-ins for environmental neurotoxicants | adjacent | High | |
| Binding of chemicals to ionotropic glutamate receptors leads to impairment of learning and memory via loss of drebrin from dendritic spines of neurons | adjacent | High | Moderate |

## Relationship: 359: Neuronal network function, Decreased leads to Impairment, Learning and memory

### AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| Nicotinic acetylcholine receptor activation contributes to abnormal roll change within the worker bee caste leading to colony loss/failure 2 | adjacent | | |
| Nicotinic acetylcholine receptor activation contributes to abnormal role change within the worker bee caste leading to colony death failure 1 | adjacent | | |
| Inhibition of Na+/I- symporter (NIS) leads to learning and memory impairment | adjacent | High | Low |
| Binding of electrophilic chemicals to SH(thiol)-group of proteins and /or to seleno-proteins involved in protection against oxidative stress during brain development leads to impairment of learning and memory | adjacent | High | |
| Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment of learning and memory abilities | adjacent | Low | |
| Binding of chemicals to ionotropic glutamate receptors leads to impairment of learning and memory via loss of drebrin from dendritic spines of neurons | adjacent | High | Moderate |

### Evidence Supporting Applicability of this Relationship

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| human | Homo sapiens | High | NCBI |
| rat | Rattus norvegicus | High | NCBI |
| mouse | Mus musculus | High | NCBI |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| During brain development | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|

| Sex | Evidence |
|---|---|
| Mixed | High |

Synaptic transmission and plasticity are achieved via mechanisms common across taxonomies. LTP has been recorded in aplysia, lizards, turtles, birds, mice, guinea pigs, rabbits and rats. Deficiencies in hippocampally based learning and memory following developmental hypothyroidism have been documented mainly in rodents and humans.

### Key Event Relationship Description

Learning and memory is one of the outcomes of the functional expression of neurons and neural networks from mammalian to invertebrates. Damage or destruction of neurons by chemical compounds during development when they are in the process of synapses formation, integration and formation of neural networks, will derange the organization and function of these networks, thereby setting the stage for subsequent impairment of learning and memory. Exposure to the potential developmental toxicants during neuronal differentiation and synaptogenesis will increase risk of functional neuronal network damage leading to learning and memory impairment.

Impairments in learning and memory are measured using behavioral techniques. It is well accepted that these alterations in behavior are the result of structural or functional changes in neurocircuitry. Functional impairments are often measured using field potentials of critical synaptic circuits in hippocampus and cortex. A number of studies have been performed in rodent models that reveal deficits in both excitatory and inhibitory synaptic transmission in the hippocampus as a result of developmental thyroid insufficiency (Wang et al., 2012; Oerbeck et al., 2003; Wheeler et al., 2011; Wheeler et al., 2015; Willoughby et al., 2014; Davenport and Dorcey, 1972; Tamasy et al., 1986; Akaike, 1991; Axelstad et al., 2008; Gilbert and Sui, 2006; Gilbert et al., 2016; Gilbert, 2011; Gilbert et al., 2016). A well-established functional readout of memory at the synaptic level is known as long-term potentiation (LTP) (i.e., a persistent strengthening of synapses based on recent patterns of activity). Deficiencies in LTP are generally regarded as potential substrates of learning and memory impairments. In rodent models where synaptic function is impaired by TH deficiencies, deficits in hippocampus-mediated memory are also prevalent (Gilbert and Sui, 2006; Gilbert et al., 2016; Gilbert, 2011; Gilbert et al., 2016).

### Evidence Supporting this KER

A number of studies have consistently reported alterations in synaptic transmission resulting from developmental TH disruption, and leading to decreased cognition.

**Biological Plausibility**

Long-term potentiation (LTP) is a long-lasting increase in synaptic efficacy and its discovery suggested that changes in synaptic strength could provide the substrate for learning and memory (reviewed in Lynch, 2004). Moreover, LTP is intimately related to the theta rhythm, an oscillation long associated with learning. Learning-induced enhancement in neuronal excitability, a measurement of neural network function, has also been shown in hippocampal neurons following classical conditioning in several experimental approaches (reviewed in Saar and Barkai, 2003).

On the other hand, memory requires the increase in magnitude of excitatory postsynaptic currents (EPSCs) to be developed quickly and to be persistent for few weeks at least without disturbing already potentiated contacts. Once again, a substantial body of evidence has demonstrated that tight connection between LTP and diverse instances of memory exist (reviewed in Lynch, 2004).

A review on Morris water maze (MWM) as a tool to investigate spatial learning and memory in laboratory rats also pointed out that the disconnection between neuronal networks rather than the brain damage of certain regions is responsible for the impairment of MWM performance. Functional integrated neural networks that involve the coordination action of different brain regions are consequently important for spatial learning and MWM performance (D'Hooge and De Deyn, 2001).

Moreover, it is well accepted that alterations in synaptic transmission and plasticity contribute to deficits in cognitive function. There are a number of studies that have linked exposure to TPO inhibitors (e.g., PTU, MMI), as well as iodine deficient diets, to changes in serum TH levels, which result in alterations in both synaptic function and cognitive behaviors (Akaike et al., 1991; Vara et al., 2002; Gilbert and Sui, 2006; Axelstad et al., 2008; Taylor et al., 2008; Gilbert, 2011; Gilbert et al., 2016), described in the indirect KER "Decrease of TH synthesis leads to learning and memory deficits".

**Empirical Evidence**

Developmental hypothyroidism reduces the functional integrity in brain regions critical for learning and memory. Neurophysiological indices of synaptic transmission of excitatory and inhibitory circuitry are impaired in the hippocampus of hypothyroid animals. Both hippocampal regions (area CA1 and dentate gyrus) exhibit alterations in excitatory and inhibitory synaptic transmission following reductions in serum TH in the pre and early postnatal period (Vara et al., 2002; Sui and Gilbert, 2003; Sui et al., 2005; Gilbert and Sui, 2006; Taylor et al., 2008; Gilbert, 2011; Gilbert et al., 2016). These alterations persist into adulthood despite a recovery to euthyroid conditions in blood. The latter observation indicates that these alterations represent permanent changes in brain function caused by transient hormones insufficiencies induced during critical window of development.

Because the adult hippocampus is involved in learning and memory, it is a brain region of remarkable plasticity. Use-dependent synaptic plasticity is critical during brain development for synaptogenesis and fine tuning of synaptic connectivity. In the adult brain, similar plasticity mechanisms underlie use-dependency that underlies learning and memory, as exhibited in LTP model of synaptic memory. Hypothyroidism during development reduces the capacity for synaptic plasticity in juvenile and adult offspring (Vara et al., 2002; Sui and Gilbert, 2003; Dong et al., 2005; Sui et al., 2005; Gilbert and Sui, 2006; Taylor et al., 2008; Gilbert, 2011; Gilbert et al., 2016). Decrease of neuronal network function and plasticity are observed coincident with deficits in learning tasks that require the hippocampus.

**- Wang et al., 2012:** This study showed that maternal subclinical hypothyroidism impairs spatial learning in the offspring, as well as the efficacy and optimal time of T4 treatment in pregnancy. Female adult Wistar rats were randomly divided into six groups: control, hypothyroid (H), subclinical hypothyroid (SCH) and SCH treated with T4, starting from GD10, GD13 and GD17, respectively, to restore normal TH levels. Results indicate that progenies of SCH and H groups demonstrated significantly longer mean latency in the water maze test (on the $2^{nd}$ training day, latency was ~83% higher in H group, and ~50% higher in SCH), and a lower amplification percentage of the amplitude (~15% lower in H group, and 12% lower in SCH), and slope of the field excitatory postsynaptic potential (fEPSP) recording (~20% lower in H group, and 17% lower in SCH), compared to control group. T4 treatment at GD10 and GD13 significantly shortened mean latency and increased the amplification percentage of the amplitude and slope of the fEPSPs of the progeny of rats with subclinical hypothyroidism. However, T4 treatment at GD17 showed only minimal effects on spatial learning in the offspring. Altogether these data indicate direct correlation between decrease of neural network function and learning and memory

deficits.

**- Liu et al., 2010** This study assessed the effects of hypothyroidism in 60 female rats who were divided into three groups: (i) maternal subclinical hypothyroidism (total thyroidectomy with T4 infusion), (ii) maternal hypothyroidism (total thyroidectomy without T4 infusion), and (iii) control (sham operated). The Morris water maze tests revealed that pups from the subclinical hypothyroidism group showed long-term memory deficits, and a trend toward short-term memory deficits.

**- Gilbert and Sui, 2006** Administration of 3 or 10 ppm PTU to pregnant and lactating dams via the drinking water from GD6 until PND30 caused a 47% and 65% reduction in serum T4, in the dams of the low and high-dose groups, respectively. Baseline synaptic transmission was impaired in PTU-exposed animals: mean EPSP slope (by ~60% with 10 ppm PTU) and population spike amplitudes (by ~70% with 10 ppm PTU) in the dentate gyrus were reduced in a dose-dependent manner in adult offspring of PTU-treated dams. High-dose animals (10 ppm) demonstrated very little evidence of learning despite 16 consecutive days of training (~5-fold higher mean latency to find the hidden platform, used as an index of learning).

**- Gilbert et al., 2016** Exposure to PTU during development produced dose-dependent reductions in mRNA expression of nerve growth factor (Ngf) in whole hippocampus of neonates. These changes in basal expression persisted to adulthood despite the return to euthyroid conditions in blood. Developmental PTU treatment dramatically reduced the activity-dependent expression of neurotrophins and related genes in neonate hippocampus and was accompanied by deficits in hippocampal-based learning (e.g., mean latency to find a hidden platform, at $2^{nd}$ trial resulted ~60% higher in rats treated with 10 ppm PTU).

**- Gilbert, 2011** Trace fear conditioning deficits to context and to cue reported in animals treated with PTU and who also displayed synaptic transmission and LTP deficits in hippocampus. Baseline synaptic transmission was impaired in PTU-exposed animals (by ~50% in animal treated with 3 ppm PTU). EPSP slope amplitudes in the dentate gyrus were reduced in a dose-dependent manner in adult offspring of PTU-treated dams.

BPA, an environmental toxicant known to inhibit NIS-mediated iodide uptake (Wu Y et al., 2016) has been found to cause learning and memory deficits in rodents as described below:

- **Jang et al., 2012** In this study, pregnant female C57BL/6 mice (F0) were exposed to BPA (0.1-10 mg/kg) from gestation day 6 to 17, and female offspring (F2) from F1 generation mice were analysed. Exposure of F0 mice to BPA (10 mg/kg) decreased hippocampal neurogenesis (~ 30% decrease of hippocampal $BrdU^+$ cells vs control) in F2 female mice. High-dose BPA (10 mg/kg) caused neurocognitive deficit (i.e., reduced memory retention) as shown by passive avoidance testing (~ 33% decrease vs control) in F2 mice. Furthermore, 10 mg/kg BPA decreased the hippocampal levels of BDNF (~ 35% lower vs control) in F2 mice. These results suggest that BPA exposure (NIS inhibitor) in pregnant mothers could decrease hippocampal neurogenesis (decreased number of neurons) and cognitive function in future generations.

In humans, the data linking these two specific KE are much more limited, but certainly clear reductions in IQ, with specific impairments in hippocampus-mediated functions have been observed.

**- Wheeler et al., 2015** This study assessed hippocampal functioning in adolescents with congenital hypothyroidism (CH), using functional magnetic resonance imaging (fMRI). 14 adolescents with CH and 14 typically developing controls (TDC) were studied. Hippocampal activation was greater for pairs than items in both groups, but this difference was only significant in TDC. When the groups were directly compared, the right anterior hippocampus was the primary region in which the TDC and CH groups differed for this pair memory effect. Results signify that adolescents with CH show abnormal hippocampal functioning during verbal memory processing, in order to compensate for the effects induced by TH deficit in the brain.

**- Wheeler et al., 2012** In this study hippocampal neuronal network function was measured based on synaptic performance using fMRI and was altered while subjects engaged in a memory task. Data showed paired word recognition deficits in adolescents with congenital hypothyroidism (N = 14; age range, 11.5-14.7 years) compared with controls (N = 15; age range, 11.2-15.5 years), with no impairment on simple word lists. Analysis of functional magnetic resonance imaging showed that adolescents with congenital hypothyroidism had both increased magnitude of hippocampal activation relative to controls and bilateral hippocampal activation when only the left was observed in controls. Furthermore, the increased activation in the congenital hypothyroidism group was correlated with the severity of the hypothyroidism experienced early in life.

**- Willoughby et al., 2013** Analogously, in this study, fMRI revealed increased hippocampus activation with word pair recognition task in CH and children born to women with hypothyroxinemia during midgestation. These differences in functional activation were not seen with single word recognition, but were revealed when retention of word pair associations was probed. The latter is a task requiring engagement of the hippocampus.

A series of important findings suggest that the biochemical changes that happen after induction of LTP also occur during memory acquisition, showing temporality between the two KEs (reviewed in Lynch, 2004).

**- Morris et al., 1986** This study found that blocking the NMDA receptor of the neuronal network with AP5 inhibits spatial learning in rats. Most importantly, in the same study they measured brain electrical activity and recorded that this agent also inhibits LTP, however, they have not proven that spatial learning and LTP inhibition are causally related.

Since then a number of NMDA receptor antagonists have been studied towards their ability to induce impairment of learning and memory. It is worth mentioning that similar findings have been found in human subjects:

**- Grunwald et al., 1999** By combining behavioural and electrophysiological data from patients with temporal lobe epilepsy exposed to ketamine, involvement of NMDA receptors in human memory processes was demonstrated.

The last KE preceding the AO (learning and memory deficits), i.e. "Decreased Neural Network Function", is also common to the AOP 13, entitled "Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment of learning and memory abilities" (https://aopwiki.org/aops/13). In this AOP 13, data on lead (Pb) exposure as reference chemical are reported. While these studies do not refer to TH disruption, they provide empirical support for the same KER described in the present AOP.

**Pb2+:** Exposure to low levels of Pb2+, during early development, has been implicated in long-lasting behavioural abnormalities and cognitive deficits in children (Needleman et al., 1975; Needleman and Gatsonis, 1990; Bellinger et al., 1991; 1992; Baghurst et al.,

1992; Leviton et al., 1993; Needleman et al., 1996; Finkelstein et al., 1998; Lanphear et al., 2000; 2005; Canfield et al., 2003; Bellinger 2004; Lanphear et al., 2005; Surkan et al., 2007; Jusko et al., 2008; Neal and Guilarte, 2010) and experimental animals (Brockel and Cory-Slechta, 1998; Murphy and Regan, 1999; Moreira et al., 2001). Multiple lines of evidence suggest that Pb2+ can impair hippocampus-mediated learning in animal models (reviewed in Toscano and Guilarte, 2005).

**- Jett et al., 1997** Female rats exposed to $Pb^{2+}$ through gestation and lactation have shown more severe impairment of memory than male rats with similar $Pb^{2+}$ exposures.

**- De Souza Lisboa et al., 2005** This study reported that exposure to $Pb^{2+}$ during both pregnancy and lactation caused depressive-like behaviour (detected in the forced swimming test) in female but not male rats.

**- Anderson et al., 2012** This study investigated the neurobehavioral outcomes in $Pb^{2+}$-exposed rats (250, 750 and 1500 ppm $Pb^{2+}$ acetate in food) during gestation and through weaning and demonstrated that these outcomes are very much influenced by sex and rearing environment. In females, $Pb^{2+}$ exposure lessened some of the benefits of enriched environment on learning, whereas, in males, enrichment does help to overcome detrimental effects of $Pb^{2+}$ on learning. Regarding reference memory, environmental enrichment has not been beneficial in females when exposure to $Pb^{2+}$ occurs, in contrast to males.

**- Jaako-Movits et al., 2005** Wistar rat pups were exposed to 0.2% $Pb^{2+}$ via their dams' drinking water from PND 1 to PND 21 and directly via drinking water from weaning until PND 30. At PND 60 and 80, the neurobehavioural assessment has revealed that developmental $Pb^{2+}$ exposure induces persistent increase in the level of anxiety and inhibition of contextual fear conditioning. The same behavioural syndrome in rats has been described in Salinas and Huff, 2002.

**- Finkelstein et al., 1998** These observations are in agreement with observations on humans, as children exposed to low levels of $Pb^{2+}$ displayed attention deficit, increased emotional reactivity and impaired memory and learning.

**- Kumar and Desiraju, 1992** In Wistar rats fed with lead acetate (400 µg/g body weight/day) from PND 2 until PND 60, EEG findings showed statistically significant reduction in the delta, theta, alpha and beta band EEG spectral power in motor cortex and hippocampus, but not in delta and beta bands power of motor cortex in wakeful state. After 40 days of recovery, animals were assessed for their neurobehaviour, and revealed that $Pb^{2+}$ treated animals showed more time and sessions in attaining criterion of learning than controls.

Further data obtained using animal behavioral techniques demonstrate that NMDA mediated synaptic transmission is decreased by $Pb^{2+}$ exposure (Cory-Slechta, 1995; Cohn and Cory-Slechta, 1993 and 1994).

**- Xiao et al., 2014** Rat pups from parents exposed to 2 mM $PbCl_2$ three weeks before mating until their weaning (pre-weaning $Pb^{2+}$) and weaned pups exposed to 2 mM $PbCl_2$ for nine weeks (post-weaning $Pb^{2+}$) were assessed for their spatial learning and memory by MWM on PND 85-90. The study revealed that both rat pups in pre-weaning $Pb^{2+}$ and post-weaning $Pb^{2+}$ groups performed significantly worse than those in the control group. The number of synapses in pre-weaning $Pb^{2+}$ group increased significantly, but it was still less than that of control group. The number of synapses in post-weaning $Pb^{2+}$ group was also less than that of control group, although the number of synapses had no differences between post-weaning $Pb^{2+}$ and control groups before MWM. In both pre-weaning $Pb^{2+}$ and post-weaning $Pb^{2+}$ groups, synaptic structural parameters such as thickness of postsynaptic density (PSD), length of synaptic active zone and synaptic curvature increased, whereas width of synaptic cleft decreased compared to controls.

**The last KE preceding the AO (learning and memory deficits), i.e. "Decreased Neural Network Function", is also common to the AOP 17, entitled " Binding of electrophilic chemicals to SH(thiol)-group of proteins and /or to seleno-proteins during brain development leads to impairment of learning and memory" (https://aopwiki.org/aops/17). In this AOP 17, data on mercury exposure as reference chemical are reported. While these studies do not refer to TH disruption, they provide empirical support for the same KER described in the present AOP.**

**Sokolowski et al. 2013**. Rats at postnatal day 7 received a single injection of methylmercury (0.6 microgr/g, that caused caspase activation in the hilus of granule cell layer in hippocampus. At PD 21, a decrease in cell number or 22% in hilus and of 27% in granule cell layer, as well as a decreased proliferation of neural precursor cells of 25% were observed. This was associated with a decrease of spatial memory as assessed by Morris water maze.

**Eddins et al., 2008**. Mice exposed during postnatal week 1-3 to 2-5 mg/kg mercury chloride in 0.01 ml/g of NaCl injectd s.c. The behavioral tests at 3 months of age revealed learning deficits (radial maze), which was associated with increased levels of monoamines in frontal cortex.

**Zanoli et al., 1994.** Single injection of methylmercury (8 mg/kg by gavage) at gestational day 15. Offsprings analyzed at 14, 21, and 60 days of age exhibited a decrease in the number of muscarinic receptors at 14 and 21 days and a decrease in avoidance latency at 60 days, indicating learning and memory deficits.

**Zanoli et al., 2001.** Single injection of methylmercury (8 mg/kg) at gestational day 8. Brain was removed at PD 21 and 60. An increase in tryptophan level in hippocampus was detected at both days. At PD 21, a decrease in anthranilic acid and an increase in quinolinic acid was found. No change in glutamic acid nor in aspartic acid were detected.

**Montgomery et al., 2008.** C57/B6 mice exposed during pregnancy (GD 8-18) with food containing methylmercury (0.01 mg/kg body wheight). Tested when adult, they showed deficits in motor function, coordination, overall activity and impairment in reference memory.

**Glover et al., 2009.** Balb mice exposed to methylmercury in diet (low dose: 1.5 mg/kg; high dose: 4.5 mg/kg) during 11 weeks (6 weeks prior mating, 3 weeks during gestation and 2 weeks post-partum). Offsprings tested at PD 15 showed an accumulation of Hg in brain (0.08 mg/kg for low dose and 0.25 mg/kg for the high dose). At hte cellular level, there was alterations in gene expression for cytoskeleton, cell processes, cell adhesion, cell differentiation, development), which could be all involved in cellular network formation. This was associated with behavioral impairment, i.e. a decrease in exploratory activity measured in open field.

**Onishchenko et al., 2007**. Pregnant mice received 0.5 mg methylmercury/kg/day in drinking water from gestational dy 7 until day 7 after delivery. Offspring behavior was monitored at 5-15 and 26-36 weeks of age. Mercury-induced alterations in reference memory were detected.

**Cagiano et al., 1990.** Pregnant rat received at GD 15 8mg/kg of methylmercury by gavage. Offsprings were tested at day 16, 21 and 60. A reduced functional activity of glutamatergic system associated with disturbances in learning and memory were observed.

**Rice, 1992.** Female monkeys exposed to 10, 25 and 50 microg/kg/day to methylmercury. Male unexposed. Infants separated from

mother at birth and exposed to similar doses did not show gross intellectual impairment, but interferences with temporal discrimination.

**Sahin et al., 2016.** Exposure of rat pups for 5 weeks or 5 months with mercury chloride (4.6 microg/kg as first injection, followed each day by 0.07 microg/kg/day). Learning and memory impairment measured by passive avoidance and Morris-water-maze was found in 5-weeks group, but not in the 5-month group. This was accompanied by hearing loss.

**In humans:**

**Orenstein et al., 2014.** Maternal peripartum hair mercury level was measured to assess prenatal mercury exposure. The concentrations of mercury was found in the range of 0.3-5.1 microg/g, similar to fish eating population in US. However, statistical analyses revealed that each microg/g increase in hair Hg was associated with a decrement in visula memory, learning and verbal memory.

**Yorifuji et al., 2011**. A survey of the Minamata exposed population made in 1971 to assess pre- and post-natal exposure revealed a methylmercury-induced impairment of intelligence as well as behavioral dysfunction.

**Uncertainties and Inconsistencies**

One of the most difficult issues for neuroscientists is to link neuronal network function to cognition, including learning and memory. It is still unclear what modifications of neuronal circuits need to happen in order to alter motor behaviour as it is recorded in a learning and memory test (Mayford et al., 2012), meaning that there is no clear understanding about how these two KEs are connected.

The direct relationship of alterations in neural network function and specific cognitive deficits is difficult to ascertain given the many forms that learning and memory can take and the complexity of synaptic interactions in even the simplest brain circuit. Linking of neurophysiological assessments to learning and memory processes have, by necessity, been made across simple monosynaptic connections and largely focused on the hippocampus. Alterations in synaptic function have been found in the absence of behavioral impairments. This may result from measuring only one component in the complex brain circuitry that underlies 'cognition', behavioral tests that are not sufficiently sensitive for the detection of subtle cognitive impairments, and behavioral plasticity whereby tasks are solved by the animal via different strategies developed as a consequence of developmental insult.

Finally, in order to provide empirical support for this KER, data on the effects of lead (Pb) exposure are reported. Several epidemiological studies where Pb2+ exposure levels have been studied in relation to neurobehavioural alterations in children have been reviewed in Koller et al. 2004. This review has concluded that in some occasions there is negative correlation between Pb2+ dose and cognitive deficits of the subjects due to high influence of social and parenting factors in cognitive ability like learning and memory (Koller et al. 2004), meaning that not always Pb2+ exposure is positively associated with learning and memory impairment in children.

**Mercury**

**Olczak et al., 2001.** Postnatal exposure of rats to Thimerosal (4 injections with 12, 240, 1440 and 3000 microgHg/kg per injection). Effects were measured in adult, which exhibited alterations in dopaminergic system with decline in the density of striatal D2 receptors, with a higher sensitivity for males. No alterations in spatial learning and memory was observed, but impairments of motor activity, increased anxiety (open fiel measurment), which are other symptoms of autism spectrum disorder.

**Franco et al., 2006.** Lactational exposure of mice to methylmercury in drinking water (10 mg/L). Analysis at weaning revealed only impairment in motor performances.

**Franco et al., 2007.** Lactational exposure of mice with mercury chloride (0.5 and 1.5 mg/kg, i.p. injection once a day).. At weaning , animals exhibited an increased level of mercury in cerebellum associated with motor deficit.

**Cardenas et al., 2017** showed that maternal red blood cell mercury of 3.8 ng/g was associated to increased DNA methylation of PON1 in umbilical cord blood only in male and observed deficit in cognitive performances, such as visual motor ability, vocabiary and verbal intellgence.

## References


Akaike M, Kato, N., Ohno, H., Kobayashi, T. (1991). Hyperactivity and spatial maze learning impairment of adult rats with temporary neonatal hypothyroidism. Neurotoxicol Teratol 13:317-322.

Anderson DW, Pothakos K, Schneider JS. (2012). Sex and rearing condition modify the effects of perinatal lead exposure on learning and memory. Neurotoxicology 33: 985-995.

Axelstad M, Hansen PR, Boberg J, Bonnichsen M, Nellemann C, Lund SP, Hougaard KS, U H. (2008). Developmental neurotoxicity of Propylthiouracil (PTU) in rats: relationship between transient hypothyroxinemia during development and long-lasting behavioural and functional changes. Toxicol Appl Pharmacol 232:1-13.

Baghurst PA, Tong S, Sawyer MG, Burns J, McMichael AJ. (1992). Environmental exposure to lead and children’s intelligence at the age of seven years. The Port Pirie Cohort Study. N Engl J Med. 327: 1279-1284.

Bellinger D, Sloman J, Leviton A, Rabinowitz M, Needleman HL, Waternaux C. (1991). Low-level lead exposure and children's cognitive function in the preschool years. Pediatrics. 87: 219-227.

Bellinger DC, Stiles KM, Needleman HL. (1992). Low-level lead exposure, intelligence and academic achievement: a long-term follow-up study. Pediatrics. 90: 855-861.

Bellinger DC. (2004). Lead. Pediatrics 113: 1016-1022.

Brockel BJ, Cory-Slechta DA. (1998). Lead, attention, and impulsive behavior: changes in a fixed-ratio waiting-for-reward paradigm. Pharmacol Biochem Behav. 60: 545-552.

Cagiano, R., et al. (1990). "Evidence that exposure to methyl mercury during gestation induces behavioral and neurochemical changes in offspring of rats." Neurotoxicol Teratol **12**(1): 23-28.

Canfield RL, Henderson CR Jr, Cory-Slechta D, Cox C, Jusko TA, Lanphear BP. (2003). Intellectual impairment in children with blood lead concentrations below 10 µg per deciliter. N Engl J Med. 348: 1517-1526.

Cao X, Huang S, Ruan D. (2008). Enriched environment restores impaired hippocampal long-term potentiation and water maze performance induced by developmental lead exposure in rats. Dev Psychobiol. 50: 307-313.

Cardenas A, Rifas-Shiman SL, Agha G, Hivert MF, Litonjua AA, DeMeo DL, Lin X, Amarasiriwardena CJ, Oken E, Gillman MW, Baccarelli AA., Persistent DNA methylation changes associated with prenatal mercury exposure and cognitive performance during childhood., Sci Rep. 2017 Mar 21;7(1):288. doi: 10.1038/s41598-017-00384-5.

Cohn J, Cory-Slechta DA. (1993). Subsensitivity of lead-exposed rats to the accuracy-impairing and rate-altering effects of MK-801 on a multiple schedule of repeated learning and performance. Brain Res. 600: 208-218.

Cohn J, Cory-Slechta DA. (1994). Lead exposure potentiates the effects of N-methyl-D-asparate on repeated learning. Neurotoxicol Teratol. 16: 455-465.

Cory-Slechta DA. (1995). MK-801 subsensitivity following postweaning lead exposure. Neurotoxicology 16: 83-95.

de Souza Lisboa S F, Gonzalves G, Komatsu F, Salci Queiroz CA, Aparecido Almeida A, Gastaldello Moreira EN. (2005). Developmental lead exposure induces depressive-like behavior in female rats. Drug Chem Toxicol. 28: 67-77.

D'Hooge R, De Deyn PP. (2001). Applications of the Morris water maze in the study of learning and memory. Brain Res Brain Res Rev. 36: 60-90.

Dong J, Yin H, Liu W, Wang P, Jiang Y, Chen J. (2005). Congenital iodine deficiency and hypothyroidism impair LTP and decrease C-fos and C-jun expression in rat hippocampus. Neurotoxicology 26:417-426.

Eddins, D., et al. (2008). "Mercury-induced cognitive impairment in metallothionein-1/2 null mice." Neurotoxicol Teratol**30**(2): 88-95.

Finkelstein Y, Markowitz ME, Rosen JF. (1998). Low-level lead-induced neurotoxicity in children: an update on central nervous system effects. Brain Res Rev. 27: 168-176.

Franco, J. L., et al. (2006). "Cerebellar thiol status and motor deficit after lactational exposure to methylmercury." Environ Res**102**(1): 22-28.

Franco, J. L., et al. (2007). "Lactational exposure to inorganic mercury: evidence of neurotoxic effects." Neurotoxicol Teratol**29**(3): 360-367.

Gilbert ME, Kelly ME, Samsam TE, Goodman JH. (2005). Chronic developmental lead exposure reduces neurogenesis in adult rat hippocampus but does not impair spatial learning. Toxicol Sci. 86: 365-374.

Gilbert ME. (2011). Impact of low-level thyroid hormone disruption induced by propylthiouracil on brain development and function. Toxicol Sci 124:432-445.

Gilbert ME, Sanchez-Huerta K, Wood C. (2016). Mild Thyroid Hormone Insufficiency During Development Compromises Activity-Dependent Neuroplasticity in the Hippocampus of Adult Male Rats. Endocrinology 157:774-787.

Gilbert ME, Sui L. (2006). Dose-dependent reductions in spatial learning and synaptic function in the dentate gyrus of adult rats following developmental thyroid hormone insufficiency. Brain Res 1069:10-22.

Glover, C. N., et al. (2009). "Methylmercury speciation influences brain gene expression and behavior in gestationally-exposed mice pups." Toxicol Sci **110**(2): 389-400.

Grunwald T, Beck H, Lehnertz K, Blümcke I, Pezer N, Kurthen M, Fernández G, Van Roost D, Heinze HJ, Kutas M, Elger CE. (1999). Evidence relating human verbal memory to hippocampal N-methyl-D-aspartate receptors. Proc Natl Acad Sci U S A. 96: 12085-12089.

Jaako-Movits K, Zharkovsky T, Romantchik O, Jurgenson M, Merisalu E, Heidmets LT, Zharkovsky A. (2005). Developmental lead exposure impairs contextual fear conditioning and reduces adult hippocampal neurogenesis in the rat brain. Int J Dev Neurosci. 23: 627-635.

Jang YJ, Park HR, Kim TH, Yang WJ, Lee JJ, Choi SY, Oh SB, Lee E, Park JH, Kim HP, Kim HS, Lee J. (2012). High dose bisphenol A impairs hippocampal neurogenesis in female mice across generations. Toxicology. Jun 14;296(1-3):73-82.

Jett DA, Kuhlmann AC, Farmer SJ, Guilarte TR. (1997). Age-dependent effects of developmental lead exposure on performance in the Morris water maze. Pharmacol Biochem Behav. 57: 271-279.

Jusko TA, Henderson CR, Lanphear BP, Cory-Slechta DA, Parsons PJ, Canfield RL. (2008). Blood lead concentrations < 10 microg/dL and child intelligence at 6 years of age. Environ Health Perspect 116:243-248.

Koller K, Brown T, Spurgeon A, Levy L. (2004). Recent developments in low-level lead exposure and intellectual impairment in children. Environ Health Perspect. 112: 987-994.

Kumar MV, Desiraju T. (1992). EEG spectral power reduction and learning disability in rats exposed to lead through postnatal developing age. Indian J Physiol Pharmacol. 36: 15-20.

Lanphear BP, Dietrich K, Auinger P, Cox C. (2000). Cognitive deficits associated with blood lead concentrations <10 microg/dL in US children and adolescents. Public Health Rep. 115: 521-529.

Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, Canfield RL, Dietrich KN, Bornschein R, Greene T, Rothenberg SJ, Needleman HL, Schnaas L, Wasserman G, Graziano J, Roberts R. (2005). Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. Environ Health Perspect. 113: 894-899.

Leviton A, Bellinger D, Allred EH, Rabinowitz M, Needleman H, Schoenbaum S. (1993). Pre- and postnatal low-level lead exposure and children's dysfunction in school. Environ Res 60:30-43.

Liu D, Teng W, Shan Z, Yu X, Gao Y, Wang S, Fan C, Wang H, Zhang H. (2010). The effect of maternal subclinical hypothyroidism during pregnancy on brain development in rat offspring. Thyroid 20:909–915.

Lynch MA. (2004). Long-term potentiation and memory. Physiol Rev. 84: 87-136.

Mayford M, Siegelbaum SA, Kandel ER. (2012). Synapses and memory storage. Cold Spring Harb Perspect Biol. 4. pii: a005751.

Montgomery, K. S., et al. (2008). "Chronic, low-dose prenatal exposure to methylmercury impairs motor and mnemonic function in adult C57/B6 mice." Behav Brain Res **191**(1): 55-61.

Moreira EG, Vassilieff I, Vassilieff VS. (2001). Developmental lead exposure: behavioral alterations in the short and long term.

Neurotoxicol Teratol. 23: 489-495.

Morris RG, Anderson E, Lynch GS, Baudry M. (1986). Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. Nature 319: 774-776.

Murphy KJ, Regan CM. (1999). Low level lead exposure in the early postnatal period results in persisting neuroplastic deficits associated with memory consolidation. J Neurochem. 72: 2099-2104.

Neal AP, Guilarte TR. (2010). Molecular Neurobiology of Lead (Pb2+): Effects on Synaptic Function. Mol Neurobiol. 42: 151-160.

Needleman HL, Epstein S, Carnow B, Scanlon J, Parkinson D, Samuels S, Mazzochi A, David O. (1975). Letter: Blood-lead levels, behaviour and intelligence. Lancet 1: 751-752.

Needleman HL, Gatsonis CA. (1990). Low-level lead exposure and the IQ of children. A meta-analysis of modern studies. Jama 263: 673-678.

Needleman HL, Riess JA, Tobin MJ, Biesecker GE, Greenhouse JB. (1996). Bone Lead Levels and Delinquent Behavior. Jama 275: 363-369.

Olczak, M., et al. (2011). "Persistent behavioral impairments and alterations of brain dopamine system after early postnatal administration of thimerosal in rats." Behav Brain Res **223**(1): 107-118.

Onishchenko, N., et al. (2007). "Developmental exposure to methylmercury alters learning and induces depression-like behavior in male mice." Toxicol Sci **97**(2): 428-437.

Orenstein, S. T., et al. (2014). "Prenatal organochlorine and methylmercury exposure and memory and learning in school-age children in communities near the New Bedford Harbor Superfund site, Massachusetts." Environ Health Perspect **122**(11): 1253-1259.

Rice, D. C. (1992). "Effects of pre- plus postnatal exposure to methylmercury in the monkey on fixed interval and discrimination reversal performance." Neurotoxicology **13**(2): 443-452.

Saar D, Barkai E. (2003). Long-term modifications in intrinsic neuronal properties and rule learning in rats. Eur J Neurosci. 17: 2727-2734.

Sahin, D., et al. (2016). "Effects of gestational and lactational exposure to low dose mercury chloride (HgCl2) on behaviour, learning and hearing thresholds in WAG/Rij rats." EXCLI J **15**: 391-402.

Salinas JA, Huff NC. (2002). Lead and conditioned fear to contextual and discrete cues. Neurotoxicol Teratol. 24: 541-550.

Sokolowski, K., et al. (2013). "Neural stem cell apoptosis after low-methylmercury exposures in postnatal hippocampus produce persistent cell loss and adolescent memory deficits." Dev Neurobiol **73**(12): 936-949.

Surkan PJ, Zhang A, Trachtenberg F, Daniel DB, McKinlay S, Bellinger DC. (2007). Neuropsychological function in children with blood lead levels <10 microg/dL. Neurotoxicology. 28: 1170-1177.

Sui L, Anderson WL, Gilbert ME. (2005). Impairment in short-term but enhanced long-term synaptic potentiation and ERK activation in adult hippocampal area CA1 following developmental thyroid hormone insufficiency. Toxicol Sci 85:647-656.

Sui L, Gilbert ME. (2003). Pre- and postnatal propylthiouracil-induced hypothyroidism impairs synaptic transmission and plasticity in area CA1 of the neonatal rat hippocampus. Endocrinology 144:4195-4203.

Taylor MA, Swant J, Wagner JJ, Fisher JW, Ferguson DC. (2008). Lower thyroid compensatory reserve of rat pups after maternal hypothyroidism: correlation of thyroid, hepatic, and cerebrocortical biomarkers with hippocampal neurophysiology. Endocrinology 149:3521-3530.

Toscano CD, Guilarte TR. (2005). Lead neurotoxicity: From exposure to molecular effects. Brain Res Rev. 49: 529-554.

Vara H, Martinez B, Santos A, Colino A. (2002). Thyroid hormone regulates neurotransmitter release in neonatal rat hippocampus. Neuroscience 110:19-28.

Wang S, Teng W, Gao Y, Fan C, Zhang H, Shan Z. (2012). Early levothyroxine treatment on maternal subclinical hypothyroidism improves spatial learning of offspring in rats. J Neuroendocrinol 24:841–848.

Wheeler SM, McAndrews MP, Sheard ED, Rovet J. (2012). Visuospatial associative memory and hippocampal functioning in congenital hypothyroidism. J Int Neuropsychol Soc 18:49-56.

Wheeler SM, McLelland VC, Sheard E, McAndrews MP, Rovet JF. (2015). Hippocampal Functioning and Verbal Associative Memory in Adolescents with Congenital Hypothyroidism. Front Endocrinol (Lausanne) 6:163.

Willoughby KA, McAndrews MP, Rovet J. (2013). Effects of early thyroid hormone deficiency on children's autobiographical memory performance. J Int Neuropsychol Soc 19:419-429.

Willoughby KA, McAndrews MP, Rovet JF. (2014). Effects of maternal hypothyroidism on offspring hippocampus and memory. Thyroid 24:576-584.

Wu Y, Beland FA1, Fang JL. (2016). Effect of triclosan, triclocarban, 2,2',4,4'-tetrabromodiphenyl ether, and bisphenol A on the iodide uptake, thyroid peroxidase activity, and expression of genes involved in thyroid hormone synthesis. Toxicol In Vitro. Apr;32:310-9.

Xiao Y, Fu H, Han X, Hu X, Gu H, Chen Y, Wei Q, Hu Q. (2014). Role of synaptic structural plasticity in impairments of spatial learning and memory induced by developmental lead exposure in Wistar rats. PLoS One. 23;9(12):e115556.

Xu J, Yan HC, Yang B, Tong LS, Zou YX, Tian Y. (2009). Effects of lead exposure on hippocampal metabotropic glutamate receptor subtype 3 and 7 in developmental rats. J Negat Results Biomed. 8: 5.

Yorifuji, T., et al. (2011). "Long-term exposure to methylmercury and psychiatric symptoms in residents of Minamata, Japan." Environ Int **37**(5): 907-913.

Zanoli, P., et al. (1994). "Methyl mercury during late gestation affects temporarily the development of cortical muscarinic receptors in rat offspring." Pharmacol Toxicol **75**(5): 261-264.

Zanoli, P., et al. (2001). "Prenatal exposure to methyl mercury in rats: focus on changes in kynurenine pathway." Brain Res Bull **55**(2): 235-238.

## List of Non Adjacent Key Event Relationships

### Relationship: 3300: Loss of drebrin leads to Impairment, Learning and memory

#### AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| Binding of chemicals to ionotropic glutamate receptors leads to impairment of learning and memory via loss of drebrin from dendritic spines of neurons | non-adjacent | High | High |

#### Evidence Supporting Applicability of this Relationship

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| Human, rat, mouse | Human, rat, mouse | High | NCBI |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| During development and at adulthood | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Mixed | Not Specified |

#### Key Event Relationship Description

Loss of drebrin disrupts dendritic spine morphology and impairs synaptic plasticity, leading to deficits in learning and memory. Experimental evidence demonstrates that drebrin reduction, achieved either through genetic deletion (up to 80–90%) or antisense knockdown, results in decreased spine density and increased spine length in hippocampal neurons. These structural abnormalities coincide with functional impairments, including reduced hippocampal synaptic plasticity, disrupted regulation of NMDA receptors or associated receptor complexes, and enhanced mGluR5-dependent long-term depression. Animal studies consistently link these molecular and cellular changes to impaired fear learning, spatial memory, and broader cognitive functions.

Implicitly, these findings suggest that drebrin is essential for maintaining proper neuronal circuitry and synaptic integrity, and that its depletion may broadly compromise neuronal network functionality and cognitive processing, even though such broader implications are not explicitly detailed in the original description of key events.

### Relationship: 3506: Binding of agonist, Ionotropic glutamate receptors leads to Loss of drebrin

#### AOPs Referencing Relationship

| AOP Name | Adjacency | Weight of Evidence | Quantitative Understanding |
|---|---|---|---|
| Binding of chemicals to ionotropic glutamate receptors leads to impairment of learning and memory via loss of drebrin from dendritic spines of neurons | non-adjacent | High | High |

#### Evidence Supporting Applicability of this Relationship

**Taxonomic Applicability**

| Term | Scientific Term | Evidence | Links |
|---|---|---|---|
| human, mouse, rat | human, mouse, rat | High | NCBI |

**Life Stage Applicability**

| Life Stage | Evidence |
|---|---|
| During brain development, adulthood and aging | High |

**Sex Applicability**

| Sex | Evidence |
|---|---|
| Mixed | Not Specified |

#### Key Event Relationship Description

Glutamate stimulation (100 µM;10 min) of cortical and hippocampal cultured neurons induced disappearance of drebrin immunostaining from dendritic spines but led to appearance of drebrin immunostaining in dendritic shafts and somata. The glutamate-induced shift of drebrin immunostaining was blocked by an NMDA receptor antagonist. Immunoblot analyses showed that both the total and the cytosolic drebrin remained unchanged and revealed that the drebrin shift was not due to drebrin degradation for the stimulation condition.