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)

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Abstract

Neurotoxicity risk assessment is an important issue for regulatory agencies. Currently, chemicals with potential risks are determined by time-consuming and costly animal testing. Therefore, *in vitro* testing methods are needed to rapidly evaluate thousands of chemicals for which no safety data on neurotoxicity exist. In recent years, chemicals that induce learning and memory impairment are thought to increase the risk of neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease. Therefore, such risk assessment is necessary for human safety. The existing AOP No. 6 in OECD Series on AOPs (AOP48 in the AOP-Wiki) defines a molecular initiating event (MIE) as “Binding of agonists to ionotropic glutamate receptors”, causing neuronal cell death linked to impairment of learning and memory through receptor hyperactivation(1).

Recent studies have shown that synaptic dysfunction precedes neuronal death in the early stages of dementia accompanied with the neurodegenerative diseases. Synaptic dysfunction is presumed as a decrease in the number of dendritic spines in neurons of the cerebral cortex and hippocampus, which are essential for learning and memory(2). Therefore, the risk for impairment of learning and memory can be assessed by the synaptic dysfunction, namely decreased number of dendritic spines(3).

Dendritic spines are small actin-rich projections protruding from the dendrites of neurons that form excitatory synapses in the cortex and hippocampus(4). Drebrin is an actin-binding protein that localizes to dendritic spines and is said to play a specific role in their formation(5). Drebrin is known to decrease in Alzheimer's disease with a high correlation to symptom stage(6,7). In low-density cultures of hippocampal neurons, the number of dendritic spines can be counted as the number of drebrin clusters with immunostaining.

We have developed an experimental protocol for low-density neuronal culture in 96-well plates and an algorithm that automatically counts the number of drebrin clusters by high-content imaging analysis(8). These protocols have been shown to be useful for screening chemicals that bind to the NMDA receptor. In fact, we have examined the toxicity of phencyclidine (PCP) and PCP-analogues and published results in a paper(9). We have developed not only the immunocytochemical protocol for *in vitro* assay using neuronal culture but also enzyme-linked immunosorbent assay (ELISA) kits to evaluate drebrin protein levels. Thus, decreased number of dendritic spines induced by chemicals can be assessed quantitatively as a loss of drebrin immunocytochemically and biochemically.

Here, we propose a new AOP with the same MIE as AOP No. 6, in which loss of drebrin as KE leads to impairment of learning and memory. Studies of genetically engineered mice have shown that drebrin deficiency is directly related to synaptic dysfunction and leads to the impairment of learning and memory, **even in the absence of neuronal cell death**(10,11,12). This is the most important distinction between the proposed AOP and the existing AOP. Measurement of drebrin expression levels in neurons with immunocytochemistry and/or ELISA is easy and high-reproducible. The new KE, loss of drebrin, will promote accumulation of data of chemicals for neurotoxicity. The proposed AOP is expected to contribute to the development of many *in vitro* test for neurotoxicity and to establish *in silico* prediction to evaluate safety of many substances for human and environments.

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

Sequence	Type	Event ID	Title	Short name
	KE	388	Overactivation, NMDARs	Overactivation, NMDARs
	KE	2078	Loss of drebrin	Loss of drebrin
	KE	1944	Synaptic dysfunction	Dysfunctional synapses
	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	Loss of drebrin		
Loss of drebrin	adjacent	Synaptic dysfunction		
Synaptic dysfunction	adjacent	Impairment, Learning and memory	High	Moderate

Overall Assessment of the AOP

References

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Appendix 1

List of MIEs in this AOP

Event: 875: Binding of agonist, Ionotropic glutamate receptors

Short Name: Binding of agonist, Ionotropic glutamate receptors

Key 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

Evidence for Perturbation by Stressor

Overview for Molecular Initiating Event

Evidence for Chemical Initiation of this Molecular Initiating Event

L-Glutamate and glycine (or D-serine) are endogenous agonists that bind to the LBD of specific NMDA receptor subunits. Here listed some known agonists for NMDA receptor, some of them are specific to the NR1 subunit and some others to the NR2 subunit (reviewed in Traynelis et al., 2010):

Specific to NR1

Glycine, l-Serine, d-Serine, l-Alanine, d-Alanine, d-Cycloserine, HA 966, (+)-(1-hydroxy-3-aminopyrrolidine-2-one,) β-Cl-d-Alanine, β-F-dl-Alanine, tri-F-dl-Alanine, ACPC, 1-aminocyclopropane-1-carboxylic acid, ACBC, 1-aminocyclobutane-1-carboxylic acid, GLYX-13.

Specific to NR2

l-Glutamate, d-Glutamate, l-Aspartate, d-Aspartate, N-Methyl-l-aspartate, N-Methyl-d-aspartate, SYM208,1 l-Homocysteinsulfinate, d-Homocysteinsulfinate, l-Homocysteate, d-Homocysteate, l-Cysteinesulfinate, l-Cysteate, d-Cysteate, Homoquinolinate, Ibotenate, (R,S)-(Tetrazol-5-yl)glycine, L-CCG-IV, (2S,3R,4S)-2-(carboxycyclopropyl)glycine, trans-ACBD, trans-1-aminocyclobutane-1,3-dicarboxylate, cis-ADA, cis-azetidine-2,4-dicarboxylic acid, trans-ADC, azetidine-2,4-dicarboxylic acid, cis-ACPD, (1R,3R)-aminocyclopentane-cis-dicarboxylate, cis-2,3-Piperidinedicarboxylic acid, (R)-NHP4G, 2-(N-hydroxylpyrazol-4-yl)glycine, (R,S)-Ethyl-NHP5G, 2-(N-hydroxypyrazol-5-yl)glycine, (R)-Propyl-NHP5G, 2-(N-hydroxypyrazol-5-yl)glycine.

Domoic acid (DomA) is structurally similar to kainic acid (KA) and both of them are analogues of the excitatory neurotransmitter L-glutamate. DomA induces excitotoxicity by an integrative action on ionotropic glutamate receptors at pre- and post-synaptic sides. DomA directly activates KA/AMPARs receptors followed by indirect activation of the NMDARs. Indeed, indirect activation of NMDARs by DomA is linked to the fact that KA and AMPA receptors activated by DomA induce increased levels of intracellular Ca2+ and Na+ which, in turn, causes endogenous glutamate release that subsequently potentiates activation of NMDARs (Berman and Murray, 1997; Berman et al., 2002; Watanabe et al., 2011). DomA has been demonstrated through both in vitro and in vivo approaches to indirectly activate the NMDARs (reviewed in Pulido et al., 2008).

Glufosinate (GLF)((RS)-2-amino-4-(hydroxy(methyl)phosphonoyl)butanoic acid, phosphinothricin) is a phosphorus containing amino acid herbicide that is naturally occurring as a component of the bacteria-derived bactericidal and fungicidal tripeptides bialaphos and phosalacine (Lanz et al., 2014). There are studies suggesting that convulsive and amnesic effects of GLF are mediated through direct binding and activation of NMDAR (Lantz et al., 2014; Matsumura et al., 2001). GLF agonist action at the NMDAR is expected to occur through direct 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.

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

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)

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(for Abstract and MIE)

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List of Key Events in the AOP

Event: 388: Overactivation, NMDARs

Short Name: Overactivation, NMDARs

Key Event Component

Process	Object	Action
NMDA glutamate receptor activity	NMDA selective 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.	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

Biological Context

Level of Biological Organization

Molecular

Cell term

Cell term

neuron

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

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).

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Event: 2078: Loss of drebrin

Short Name: Loss of drebrin

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

Event: 1944: Synaptic dysfunction

Short Name: Dysfunctional synapses

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

Biological Context

Level of Biological Organization

Cellular

List of Adverse Outcomes in this AOP

Event: 341: Impairment, Learning and memory

Short Name: Impairment, Learning and memory

Key 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: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:442 - Inhibition of voltage gate sodium channels leading to impairment in learning and memory during development	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

Biological Context

Level of Biological Organization

Individual

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links

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

Life Stage Applicability

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

Sex Applicability

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).

Key Event Description

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 behaviour. On the other hand, non-associative learning can be defined as an alteration in the behavioural 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 characterised 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 neuronal 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 behavioural 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, 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.

1) RAM, Barnes, MWM 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).

2) 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).

3) 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).

4) 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).

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:

1) 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).

2) 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).

3) 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).

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

5) 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).

6) 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).

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 behaviour. 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).

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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

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.

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Relationship: 2806: Overactivation, NMDARs 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		

Relationship: 2807: Loss of drebrin 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		

Relationship: 2808: Dysfunctional synapses 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	adjacent	High	Moderate