

OECD Series on Adverse Outcome Pathways No. 11

Adverse Outcome Pathway on binding to the picrotoxin site of ionotropic GABA receptors leading to epileptic seizures in adult brain

Ping Gong,

Edward J. Perkins

https://dx.doi.org/10.1787/9226875e-en

OECD

Adverse Outcome Pathway on Binding to the picrotoxin site of ionotropic GABA receptors leading to epileptic seizures in adult brain

Series on Adverse Outcome Pathways No. 11

Foreword

This Adverse Outcome Pathway (AOP) on Binding to the picrotoxin site of ionotropic GABA receptors leading to epileptic seizures in adult brain, has been developed under the auspices of the OECD AOP Development Programme, overseen by the Extended Advisory Group on Molecular Screening and Toxicogenomics (EAGMST), which is an advisory group under the Working Group of the National Coordinators for the Test Guidelines Programme (WNT). The AOP has been reviewed internally by the EAGMST, externally by experts nominated by the WNT, and has been endorsed by the WNT and the Working Party on Hazard Assessment (WPHA) in May 2019.

Through endorsement of this AOP, the WNT and the WPHA express confidence in the scientific review process that the AOP has undergone and accept the recommendation of the EAGMST that the AOP be disseminated publicly. Endorsement does not necessarily indicate that the AOP is now considered a tool for direct regulatory application.

The Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology agreed to declassification of this AOP on 26 June 2019.

This document is being published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology.

The outcome of the internal and external reviews are publicly available respectively in the AOP Wiki and the eAOP Portal of the AOP Knowledge Base at the following links: [internal review] [external review].

Table of contents

Foreword **2**

Adverse Outcome Pathway on Binding to the picrotoxin site of ionotropic GABA receptors leading to epileptic seizures in adult brain **5**

Short Title: Blocking iGABA receptor ion channel leading to seizures 5
Authors: 5

Abstract **6**

Background **7**

Graphical Representation **8**

Summary of the AOP **9**

Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO) 9
Key Event Relationships 9
Stressors 10

Overall Assessment of the AOP **12**

Biological plausibility 12
Concordance of dose-response relationships 12
Temporal concordance among the key events and the adverse outcome 12
Strength, consistency, and specificity of association of adverse effect and initiating event 13
Uncertainties, inconsistencies, and data gaps 13

Domain of Applicability **14**

Life Stage Applicability 14
Taxonomic Applicability 14
Sex Applicability 14

Essentiality of the Key Events **15**

Weight of Evidence Summary **15**

Quantitative Consideration **16**

Considerations for Potential Applications of the AOP (optional) **17**

References 17

Appendix 1 ..... **20**

List of MIEs in this AOP 20
Event: 667: Binding at picrotoxin site, iGABAR chloride channel 20
List of Key Events in the AOP 26
Event: 64: Reduction, Ionotropic GABA receptor chloride channel conductance 26
Event: 669: Reduction, Neuronal synaptic inhibition 29
Event: 682: Generation, Amplified excitatory postsynaptic potential (EPSP) 31
Event: 616: Occurrence, A paroxysmal depolarizing shift 34
List of Adverse Outcomes in this AOP 37
Event: 613: Occurrence, Epileptic seizure 37

Appendix 2 41

List of Adjacent Key Event Relationships 41

Relationship: 666: Binding at picrotoxin site, iGABAR chloride channel leads to Reduction, Ionotropic GABA receptor chloride channel conductance 41

Relationship: 667: Reduction, Ionotropic GABA receptor chloride channel conductance leads to Reduction, Neuronal synaptic inhibition 44

Relationship: 683: Reduction, Neuronal synaptic inhibition leads to Generation, Amplified excitatory postsynaptic potential (EPSP) 47

Relationship: 684: Generation, Amplified excitatory postsynaptic potential (EPSP) leads to Occurrence, A paroxysmal depolarizing shift 52

Relationship: 630: Occurrence, A paroxysmal depolarizing shift leads to Occurrence, Epileptic seizure 55

Adverse Outcome Pathway on Binding to the picrotoxin site of ionotropic GABA receptors leading to epileptic seizures in adult brain

Short Title: Blocking iGABA receptor ion channel leading to seizures

Authors:

Ping Gong, Edward J. Perkins, US Army Engineer Research and Development Center

Email: ping.gong@usace.army.mil or edward.j.perkins@usace.army.mil

Point of contact for this AOP entry: Dr. Ping Gong

Abstract

This AOP begins with a molecular initiating event (MIE) where a chemical binds to the picrotoxin binding site at or near the central pore of the ionotropic GABA receptor complex causing blockage of the ion channel. As a result, the first key event (KE) is a decrease in inward chloride conductance through the ligand-gated ion channel. This leads to the second KE, a reduction in postsynaptic inhibition, reflected as reduced frequency and amplitude of spontaneous inhibitory postsynaptic current (sIPSC) or abolishment of GABA-induced firing action in GABAergic neuronal membranes. Consequently, the resistance of excitatory neurons to fire is decreased, resulting in the generation of a large excitatory postsynaptic potential (EPSP), i.e., the third KE. The large EPSP is reflected as a spike (rise) of intracellular Ca2+ observed in the affected region, where a large group of excitatory neurons begin firing in an abnormal, excessive, and synchronized manner. Such a giant Ca2+-mediated excitatory firing (depolarization) causes voltage-gated Na+ to open, which results in action potentials. The depolarization is followed by a period of hyper-polarization mediated by Ca2+-dependent K+ channels or GABA-activated Cl− influx. During seizure development, the post-depolarization hyperpolarization becomes smaller, gradually disappears, and is replaced by a depolarization. This characteristic depolarization-shrinking hyperpolarization sequence of events represents the fourth KE known as “paroxysmal depolarizing shift” (PDS), which forms a “seizure focus”. A PDS is, essentially, an indication of epilepsy at the cellular level, which serves as the foci to initiate the adverse outcome at the organismal level of epileptic seizure. The severity of symptoms is often dose- and duration- dependent, while the toxicological symptoms are associated with the type and location of affected iGABARs. Mortality can occur if the individual sustains a prolonged or pronounced convulsion or seizure. Neurotoxicity, of which seizures are an end point, is a regulated outcome for chemicals. This AOP allows for screening chemicals for the potential to cause neurotoxicity through the use of in vitro assays that demonstrate binding to the picrotoxin site, electrophysiological assays demonstrating depolarization of neuronal membranes, or electroencephalography that records electrical activity of the adult brain.

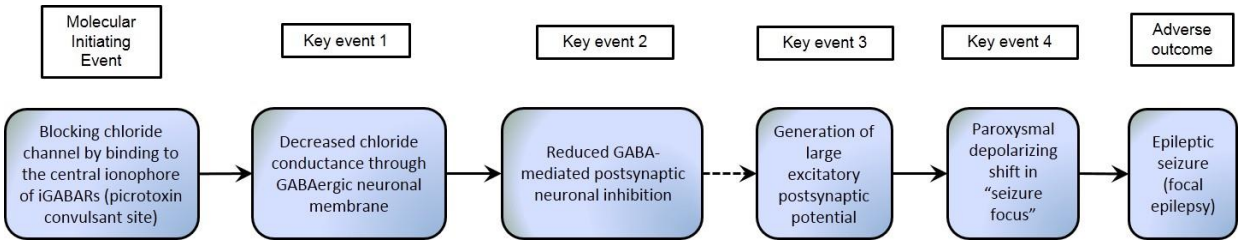
Background

Ionotropic GABA receptors (iGABARs) are ligand-gated ion channels which play important functional roles in the nervous system. As the major player in inhibitory neurotransmission, iGABARs are widely distributed in both vertebrates and invertebrates (McGonigle and Lummis 2010; Garcia-Reyero et al. 2011). In vertebrates, the iGABAR includes two subclasses of fast-responding ion channels, GABAA receptor (GABAA-R) and GABAC receptor (GABAC-R). Invertebrate iGABARs do not readily fit the vertebrate GABAA/GABAC receptor categories (Sieghart 1995). The majority of insect iGABARs are distinguished from vertebrate GABAA receptors by their insensitivity to bicuculline and differ from GABAC-Rs in that they are subject to allosteric modulation, albeit weakly, by benzodiazepines and barbiturates (Hosie et al. 1997).

Chemical interactions with iGABARs can cause a variety of pharmacological and neurotoxicological effects depending on the location of the active or allosteric site affected. Three distinct types of interactions at binding sites on iGABARs can antagonize the postsynaptic inhibitory functions of GABA and lead to epileptic seizures and death. These three types of interactions correspond to three AOPs (Gong et al. 2015). One of the three types of interaction is non-competitive channel blocking at the picrotoxin convulsant site located inside of the iGABAR pore that spans neuronal cell membranes, which is the MIE described in the current AOP. The other two types of interactions are negative modulation at allosteric sites and competitive binding at the active orthosteric sites (MIEs to be developed in the future).

It is worth noting that there exist another class of GABA receptors called metabotropic G-protein-coupled receptors (mGABAR) or $GABA_B$ receptors. This AOP is not applicable to $GABA_B$ receptors because they mediate slow and sustained inhibitory responses to GABA and are involved in absence epilepsy (Han et al. 2012).

Graphical Representation



Summary of the AOP

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

Sequence	Type	Event ID	Title	Short name
1	MIE	667	Binding at picrotoxin site, iGABAR chloride channel	Binding at picrotoxin site, iGABAR chloride channel
2	KE	64	Reduction, Ionotropic GABA receptor chloride channel conductance	Reduction, Ionotropic GABA receptor chloride channel conductance
3	KE	669	Reduction, Neuronal synaptic inhibition	Reduction, Neuronal synaptic inhibition
4	KE	682	Generation, Amplified excitatory postsynaptic potential (EPSP)	Generation, Amplified excitatory postsynaptic potential (EPSP)
5	KE	616	Occurrence, A paroxysmal depolarizing shift	Occurrence, A paroxysmal depolarizing shift
6	AO	613	Occurrence, Epileptic seizure	Occurrence, Epileptic seizure

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Binding at picrotoxin site, iGABAR chloride channel	adjacent	Reduction, Ionotropic GABA receptor chloride channel conductance	High	High
Reduction, Ionotropic GABA receptor chloride channel conductance	adjacent	Reduction, Neuronal synaptic inhibition	High	High
Reduction, Neuronal synaptic inhibition	adjacent	Generation, Amplified excitatory postsynaptic potential (EPSP)	High	Moderate
Generation, Amplified excitatory	adjacent	Occurrence, A paroxysmal depolarizing shift	Moderate	Moderate

postsynaptic potential (EPSP)				
Occurrence, A paroxysmal depolarizing shift	adjacent	Occurrence, Epileptic seizure	High	Moderate

Stressors

Name	Evidence
Picrotoxin	High
Lindane	High
Dieldrin	High
Heptachlor	High
Endosulfan	High
RDX	High
Fipronil	High

1. Picrotoxin

Picrotoxin seizures are well defined mechanistically. They arise from $GABA_A$ receptor chloride channel blockage. (See Page 131 in "Models of Seizures and Epilepsy", edited by A. Pitkanen, P.A. Schwartzkroin, S.L. Moshe. Elsevier Academic Press. 2006)

As picrotoxin effectively inhibits chloride influx in $GABA_A$ and other ionotropic receptors, it represents a universal "reference" channel blocker with whom other ligands may be compared. (A.V. Kalueff. 2007. Mapping convulsants' binding to the GABA-A receptor chloride ionophore: a proposed model for channel binding sites. Neurochem Int. 50(1): 61-8.)

2. Lindane

Neurotoxic pesticides, such as lindane, alpha-endosulfan and dieldrin, share structural similarities (and compete for the binding site) with picrotoxin, inhibit TBPS binding, induce seizures and block Cl-currents through the ionophore. (A.V. Kalueff. 2007. Mapping convulsants' binding to the GABA-A receptor chloride ionophore: a proposed model for channel binding sites. Neurochem Int. 50(1): 61-8.)

3. Dieldrin

See evidence text for picrotoxin and lindane.

4. Heptachlor

Organochlorine insecticides, including DDT, dieldrin, heptachlor, lindane, and β-hexachlorocyclohexane (β-HCH), have varied targets and mechanisms of action within the central nervous system that affect the function of sodium and calcium channels and transporters. They also interfere with γ-aminobutyric acid (GABA) neurotransmission by blocking specific GABA receptors, contributing to their neurotoxic effects (Narahashi et al., 1995).

5. Endosulfan

See "Toxicological Profile for Endosulfan" (Agency for Toxic Substances and Disease Registry, 2000). Dose- and time-dependent correlations between picrotoxin site binding

by chlorinated pesticides and convulsions were observed in mice (Cole and Casida 1986), whereas poisoning with the organochlorine insecticide endosulfan caused seizure, status epilepticus, or refractory status epilepticus in humans (Durukan et al. 2009; Moon et al. 2017; Moses and Peter 2010; Parbhu et al. 2009; Roberts et al. 2004), and eventually led to the death of a farmer (Roberts et al. 2004) and a toddler (Parbhu et al. 2009).

6. RDX

See Williams et al. (2011).

7. Fipronil

See Chen *et al*. (2006).

Overall Assessment of the AOP

Biological plausibility

The biological mechanisms underlying epilepsy (defined as a disorder of the central nervous system characterized by recurrent seizures unprovoked by an acute systemic or neurologic insult) have been investigated for more than six decades and are well understood except for a few intermediate details (Bromfield *et al*. 2006; Lomen-Hoerth and Messing 2010). As one of the cellular mechanisms of action, blocking postsynaptic GABA-mediated inhibition can lead to epileptic seizure (Dichter and Ayala 1987; Gong *et al*. 2015). It has been extensively documented that non-competitive ion channel blockers such as picrotoxin, lindane, α-endosulfan and fipronil act through binding to iGABARs (Chen *et al*. 2006; Casida and Durkin 2015). Despite large structural diversity, it has been postulated that these blockers fit a single binding site in the chloride channel lumen lined by five TM2 (TransMembrane domain 2) segments, which was supported in the β3 homopentamer by mutagenesis, pore structure studies, ligand binding, and molecular modeling (Chen *et al*. 2006; Casida and Durkin 2015). The downstream cascading key events of this AOP have also been reviewed in multiple publications (e.g., Dichter and Ayala 1987; Bromfield *et al*. 2006; Lomen-Hoerth and Messing 2010). Based on the extensive evidence supporting the MIE, KEs and the AO, there is a high likelihood and certainty that such GABA antagonists as non-competitive channel blockers produce seizures in both invertebrates and vertebrates that possess GABAergic inhibitory neurotransmission in central nervous systems (Treiman 2001; Raymond-Delpech *et al*. 2005).

Concordance of dose-response relationships

Numerous pharmacological studies have reported quantitative dose-response relationships between the dose of non-competitive antagonists and the recorded electrophysiological response of epileptic seizures. See examples for picrotoxin (Newland and Cull-Candy 1992; Ikeda 1998; Stilwell *et al*. 2006), RDX (Williams *et al*. 2011) and dieldrin (Babot *et al*. 2007; Ikeda 1998).

Temporal concordance among the key events and the adverse outcome

Given that the basic mechanism of neuronal excitability is the action potential, a hyperexcitable state can result from many causes including decreased inhibitory neurotransmission (KE2). Moreover, action potentials occur due to depolarization of the neuronal membrane, with membrane depolarization propagating down the axon to induce neurotransmitter release at the axon terminal. The action potential occurs in an all-or-none fashion as a result of local changes in membrane potential brought about by net positive inward ion fluxes. Membrane potential thus varies with the activation of ligand-gated channels, whose conductance is affected by binding to neurotransmitters. For instance, the conductance is decreased (KE1) due to the binding at allosteric sites in the chloride channel of iGABAR by non-competitive blockers (MIE).

Seizure initiation: The hypersynchronous discharges that occur during a seizure may begin in a very discrete region of the cortex and then spread to neighboring regions. Seizure initiation is characterized by two concurrent events: 1) high-frequency bursts of action potentials, and 2) hypersynchronization of a neuronal population. The synchronized bursts from a sufficient number of neurons result in a so-called spike discharge on the EEG (electroencephalogram), i.e., amplified excitatory postsynaptic potential (KE3). At the level of single neurons, epileptiform activity consists of sustained neuronal depolarization resulting in a burst of action potentials, a plateau-like depolarization associated with completion of the action potential burst, and then a rapid repolarization followed by hyperpolarization. This sequence is called the paroxysmal depolarizing shift (KE4).

Seizure propagation (AO), the process by which a partial seizure spreads within the brain, occurs when there is sufficient activation to recruit surrounding neurons. This leads to a loss of surround inhibition and spread of seizure activity into contiguous areas via local cortical connections, and to more distant areas via long association pathways such as the corpus callosum. The propagation of bursting activity is normally prevented by intact hyperpolarization and a region of surrounding inhibition created by inhibitory neurons. With sufficient activation there is a recruitment of surrounding neurons via a number of mechanisms. The above description is excerpted and summarized from Bromfield *et al.* (2006).

Strength, consistency, and specificity of association of adverse effect and initiating event

Drug- or chemical-induced focal or generalized seizures are not limited to any specific group of chemical structures, neuroreceptors or taxonomy. This AOP addresses a specific group of chemicals that are capable of binding to the picrotoxin convulsant site of iGABARs, leading to epileptic seizures. Literature evidence strongly and consistently supports such a forward association, i.e., binding to the picrotoxin site leads to epileptic seizures (see reviews Gong *et al*. 2015; Bromfield *et al*. 2006; Raymond-Delpech *et al*. 2005; Treiman 2001; Dichter and Ayala 1987). For instance, dose- and time-dependent correlations between picrotoxin site binding by chlorinated pesticides and convulsions were observed in mice (Cole and Casida 1986), whereas poisoning with the organochlorine insecticide endosulfan caused seizure, status epilepticus, or refractory status epilepticus in humans (Durukan *et al*. 2009; Moon *et al*. 2017; Moses and Peter 2010; Parbhu *et al*. 2009; Roberts *et al*. 2004), and eventually led to the death of a farmer (Roberts *et al*. 2004) and a toddler (Parbhu *et al*. 2009).

Uncertainties, inconsistencies, and data gaps

No inconsistencies have been reported so far, though some uncertainties and data gaps do exist. For instance, the process by which seizures typically end, usually after seconds or minutes, and what underlies the failure of this spontaneous seizure termination in the life-threatening condition known as status epilepticus are less well understood (Bromfield *et al*. 2006). The spread of epileptic activity throughout the brain, the development of primary generalized epilepsy, the existence of “gating", mechanisms in specific anatomic locations, and the extrapolation of hypotheses derived from simple models of focal epilepsy to explain more complex forms of epilepsies observed in human and other animals, all are also not

yet fully understood (Dichter and Ayala 1987). The remarkable plasticity of GABAergic neurons and iGABARs (e.g., iGABAR regulation by phosphorylation, and expression of potassium chloride cotransporters (KCCs) or sodium dependent anion exchangers (NDAE)) in response to insults and injury constitutes additional complexity and creates another layer of uncertainties in the emergence of epileptic seizures (Ben-Ari 2006; Galanopoulou 2008; Scharfman and Brooks-Kayal 2014).

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Adults	High

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI
mouse	Mus musculus	High	NCBI
rat	Rattus norvegicus	High	NCBI
bobwhite quail	Colinus virginianus	High	NCBI
zebrafish	Danio rerio	Moderate	NCBI

Sex Applicability

Sex	Evidence
Male	High
Female	High

This AOP is applicable to all vertebrates and invertebrates possessing iGABARs, without restrictions pertaining to sex and taxonomy. This AOP may not be applicable to young animals during their embryonic and early developmental stages when GABA acts as an excitatory neurotransmitter due to increased intracellular Cl^- concentration in immature or developing neurons (Taketo and Yoshioka 2000; Galanopoulou 2008; Ben-Ari 2006). A key feature of the immature type function of $GABA_A$ receptors is the depolarizing signaling, attributed to the inability of young neurons to maintain low intracellular chloride. The regulation of GABAergic switch is different in neurons with depolarizing vs hyperpolarizing GABAergic signaling. In mature neurons, recurrent and prolonged seizures may trigger a pathological reemergence of immature features of $GABA_A$ receptors,

which compromises the efficacy of GABA-mediated inhibition. In immature neurons with depolarizing GABAergic signaling, the physiological and pathological regulation of this system is completely different, possibly contributing to the different outcomes of early life seizures (Galanopoulou 2008).

Essentiality of the Key Events

The MIE, four key events and resulted adverse outcome listed for this AOP are all essential based on current knowledge and understanding of the structure, pharmacology, localization, classification of ionotropic GABA receptors (e.g., $GABA_A$ receptors) (Olsen 2015; Olsen and Sieghart 2009), the basic neurophysiology, neurochemistry and cellular mechanisms underlying epilepsies (Dichter and Ayala 1987; Bromfield *et al*. 2006), and the pathophysiology of seizures (Lomen-Hoerth and Messing 2010).

Weight of Evidence Summary

A novel subject matter expertise driven approach was developed for weight of evidence (WoE) assessment (Collier *et al*. 2016). This approach, tailored toward the needs of AOPs and in compliance with the AOP Users' Handbook (OECD 2017), was based on criteria and metrics related to data quality and causality (i.e., the strength of causal linkage between key events). The methodology consists of three main steps: (1) assembling evidence (preparing the AOP), (2) weighting evidence (criteria weighting and scoring), and (3) weighting the body of evidence (aggregating lines of evidence). We adopted the General Assessment Factors (GAF) established by the US EPA as the criteria for data quality evaluation, and a set of five criteria known as Bradford Hill criteria to measure the strength of causal linkages (see Table below). The numerical scoring scale corresponds to the descriptive scoring scale recommended in the Users' Handbook as follows: 4 or 5 is equivalent to High, 3 is equivalent to Moderate, and 1 or 2 is equivalent to Low. Detailed description on, supporting evidence for and quantitative understanding of each KE/KER can be found by clicking the link on each KE/KER above.

The authors of Collier *et al*. (2016), who served as the developers for several AOPs (including this one), represented subject matter experts, and they applied their expertise and best professional judgment to assign weights to the criteria and scores to each line of evidence. Final criteria scoring represented the consensus scores agreed upon after debates among the authors. For example, the MIE has been intensively reviewed where numerous documented studies provided supporting evidence. Hence, the MIE received high scores for all five GAF criteria. However, the Bradford Hill criteria connecting KE2-->KE3 and KE3-->KE4 received relatively lower scores because there still exist knowledge gaps in

the spread of epileptic activity throughout the normal CNS and the mechanism underlying the generalized epilepsies. The following table shows the results of our WoE assessment (note that scores may be inexact due to rounding).

Please note that this table and scoring system might not be consistent with the Users' Handbook.

		MIE		KE 1		KE 2		KE 3		KE 4		AO	
Data Quality Criteria	**Weight**	Score	W.S.	Score	W.S.	Score	W.S.	Score	W.S.	Score	W.S.	Score	W.S.
Soundness	31%	5	1.55	5	1.55	5	1.55	4	1.24	4	1.24	5	1.55
Applicability and Utility	19%	5	0.95	5	0.95	5	0.95	4	0.76	4	0.76	5	0.95
Clarity and Completeness	16%	5	0.8	5	0.8	5	0.8	3	0.48	3	0.48	4	0.64
Uncertainty and Variability	18%	5	0.9	5	0.9	5	0.9	3	0.54	3	0.54	4	0.72
Evaluation and Review	16%	5	0.8	5	0.8	5	0.8	5	0.8	4	0.64	4	0.64
Line of Evidence Subtotals			5		5		5		3.82		3.66		4.5
Data Quality Total	Total Raw Score		**27**								Total Normalized Score		**4.50**

		MIE to KE 1		KE 1 to KE 2		KE 2 to KE 3		KE 3 to KE 4		KE 4 to AO	
Causal Linkage Criteria	**Weight**	Score	W.S.	Score	W.S.	Score	W.S.	Score	W.S.	Score	W.S.
Biological Concordance	24%	5	1.2	5	1.2	4	0.96	4	0.96	5	1.2
Essentiality of Key Events	29%	5	1.45	5	1.45	4	1.16	3	0.87	4	1.16
Concordance of Empirical Observations	22%	4	0.88	5	1.1	3	0.66	3	0.66	3	0.66
Consistency	13%	5	0.65	5	0.65	4	0.52	3	0.39	5	0.65
Analogy	12%	5	0.6	5	0.6	4	0.48	3	0.36	5	0.6
Line of Evidence Subtotals			4.78		5		3.78		3.24		4.27
Causal Linkage Total	Total Raw Score		**21.1**							Total Normalized Score	**4.21**
Total Weight of Evidence Score	**8.71**	out of 10									

Quantitative Consideration

Many studies have reported quantitative relationships between chemicals such as drugs and pesticides and electrophysiological response. For instance, long-term exposure of primary cerebellar granule cell cultures to 3 µM dieldrin reduced the $GABA_A$ receptor function to 55% of control, as measured by the GABA-induced $^{36}Cl^-$ uptake (Babot *et al*. 2007). Juarez *et al*. (2013) observed that picrotoxin exerted concentration-dependent and reversible inhibition of GABA-induced membrane currents in primary cultured neurons obtained from the guinea-pig small intestine. The stepwise qualitative relationships between consecutive events (MIE, KEs and AO) are well established but quantitative ones are rarely documented.

Considerations for Potential Applications of the AOP (optional)

This AOP can be used to establish the mode of neurotoxicological actions for chemicals capable of binding to the picrotoxin convulsant site of iGABARs. It can also be applied to risk assessment where AOP can assist in predictive modeling of chemical toxicity. Chemicals acting through this AOP can be distinguished from neurotoxicants acting on other types of iGABAR sites (e.g., orthosteric or allosteric binding sites) or other types of neuroreceptors (e.g., ardrenergic, dopaminergic, glutaminergic, cholinergic and serotonergic receptors). More information relevant to this topic can be found in Gong et al. (2015).

References

Babot Z, Vilaro M T, Sunol C. (2007) Long-term exposure to dieldrin reduces gamma-aminobutyric acid type A and N-methyl-D-aspartate receptor function in primary cultures of mouse cerebellar granule cells. J Neurosci Res, 85(16):3687-3695.

Ben-Ari Y. (2006) Seizures beget seizures: the quest for GABA as a key player. Crit Rev Neurobiol. 18(1-2):135-44.

Bromfield EB, Cavazos JE, Sirven JI. (2006) Chapter 1, Basic Mechanisms Underlying Seizures and Epilepsy. In: An Introduction to Epilepsy [Internet]. West Hartford (CT): American Epilepsy Society; Available from: http://www.ncbi.nlm.nih.gov/books/NBK2510/.

Casida JE, Durkin KA. (2015) Novel GABA receptor pesticide targets. Pesticide Biochem Physiol. 121:22-30.

Chen L, Durkin KA, Casida J. (2006) Structural model for gamma-aminobutyric acid receptor noncompetitive antagonist binding: widely diverse structures fit the same site. Proc Natl Acad Sci USA. 103(13):5185-5190.

Cole LM, Casida JE. (1986) Polychlorocycloalkane insecticide-induced convulsions in mice in relation to disruption of the GABA-regulated chloride ionophore. Life Sci. 39 (20):1855-62.

Collier ZA, Gust KA, Gonzalez-Morales B, Gong P, Wilbanks MS, Linkov I, Perkins EJ. (2016) A weight of evidence assessment approach for adverse outcome pathways. Regul Toxicol Pharmacol. 75:46-57.

Dichter MA, Ayala GF. (1987) Cellular mechanisms of epilepsy: A status report. Science 237: 157-164.

Durukan P, Ozdemir C, Coskun R, Ikizceli I, Esmaoglu A, Kurtoglu S, Guven M. (2009) Experiences with endosulfan mass poisoning in rural areas. Eur J Emerg Med. 16(1):53-6.

Galanopoulou AS. (2008) $GABA_A$ Receptors in Normal Development and Seizures: Friends or Foes? Curr Neuropharmacol. 6(1): 1–20.

Garcia-Reyero N, Habib T, Pirooznia M, Gust KA, Gong P, Warner C, Wilbanks M, Perkins E. (2011) Conserved toxic responses across divergent phylogenetic lineages: a meta-analysis of the neurotoxic effects of RDX among multiple species using toxicogenomics. Ecotoxicology. 20(3):580-94.

Gong P, Hong H, Perkins EJ. (2015) Ionotropic GABA receptor antagonism-induced adverse outcome pathways for potential neurotoxicity biomarkers. Biomarkers in Medicine 9(11):1225-39.

Han HA, Cortez MA, Snead OC III. 2012. $GABA_B$ Receptor and Absence Epilepsy. In: Noebels JL, Avoli M, Rogawski MA, et al., editors. Jasper's Basic Mechanisms of the Epilepsies [Internet]. 4th edition. Bethesda (MD): National Center for Biotechnology Information (US); 2012. Available from: https://www.ncbi.nlm.nih.gov/books/NBK98192/.

Hosie AM, Aronstein K, Sattelle DB, ffrench-Constant RH. (1997) Molecular biology of insect neuronal GABA receptors. Trends Neurosci. 20(12): 578-583.

Ikeda T, Nagata K, Shono T, Narahashi T. (1998) Dieldrin and picrotoxinin modulation of GABA(A) receptor single channels. Neuroreport 9(14):3189-3195.

Juarez EH, Ochoa-Cortes F, Miranda-Morales M, Espinosa-Luna R, Montano L M, Barajas-Lopez C. (2013) Selectivity of antagonists for the Cys-loop native receptors for ACh, 5-HT and GABA in guinea-pig myenteric neurons. Auton Autacoid Pharmacol, 34(1-2):1-8.

Lomen-Hoerth C, Messing RO. (2010) Chapter 7: Nervous system disorders. In: Stephen J. McPhee, and Gary D. Hammer (Eds), Pathophysiology of disease: an introduction to clinical medicine (6th Edition). New York: McGraw-Hill Medical. ISBN 9780071621670.

McGonigle I, Lummis SC. (2010) Molecular characterization of agonists that bind to an insect GABA receptor. Biochemistry. 49(13):2897-902.

Moon JM, Chun BJ, Lee SD. (2017) In-hospital outcomes and delayed neurologic sequelae of seizure-related endosulfan poisoning. Seizure. 51:43-49.

Moses V, Peter JV. (2010) Acute intentional toxicity: endosulfan and other organochlorines. Clin Toxicol (Phila). 48(6):539-44.

Newland CF, Cull-Candy SG. (1992) On the mechanism of action of picrotoxin on GABA receptor channels in dissociated sympathetic neurones of the rat. J Physiol, 447: 191–213.

OECD. (2017) Users' Handbook Supplement to the Guidance Document for Developing and Assessing AOPs. OECD Environment Directorate, Environment, Health and Safety Division, Series on Testing & Assessment No. 233, Series on Adverse Outcome Pathways No. 1, ENV/JM/MONO(2016)12, Paris, France.

Olsen RW. (2015) Allosteric ligands and their binding sites define γ-aminobutyric acid (GABA) type A receptor subtypes. Adv Pharmacol. 73:167-202.

Olsen RW, Sieghart W. (2009) GABA A receptors: subtypes provide diversity of function and pharmacology. Neuropharmacology. 56(1):141-8.

Parbhu B, Rodgers G, Sullivan JE. (2009) Death in a toddler following endosulfan ingestion. Clin Toxicol (Phila). 47(9):899-901.

Raymond-Delpech V, Matsuda K, Sattelle BM, Rauh JJ, Sattelle DB. (2005) Ion channels: molecular targets of neuroactive insecticides. Invert Neurosci, 5(3-4):119-133.

Roberts DM, Dissanayake W, Sheriff MHR, Eddleston M. (2004) Refractory status epilepticus following self-poisoning with the organochlorine pesticide endosulfan. J Clinical Neurosci. 11(7): 760-2.

Scharfman HE, Brooks-Kayal AR. (2014) Is Plasticity of GABAergic Mechanisms Relevant to Epileptogenesis? In: Scharfman HE and Buckmaster PS (eds.), Issues in Clinical Epileptology: A View from the Bench, Advances in Experimental Medicine and Biology 813, pp.133-150.

Sieghart W. (1995) Structure and pharmacology of gamma-aminobutyric acid A receptor subtypes. Pharmacol.Rev. 47(2):181-234.

Stilwell GE, Saraswati S, Littleton JT, Chouinard SW. (2006) Development of a Drosophila seizure model for in vivo high-throughput drug screening. Eur J Neurosci, 24(8):2211-22.

Taketo M, Yoshioka T (2000) Developmental change of GABA(A) receptor-mediated current in rat hippocampus. Neuroscience 96(3):507-514.

Treiman DM. (2001) GABAergic mechanisms in epilepsy. Epilepsia, 42(Suppl. 3):8–12.

Williams LR, Aroniadou-Anderjaska V, Qashu F, Finne H, Pidoplichko V, Bannon D I et al. (2011) RDX binds to the GABA(A) receptor-convulsant site and blocks GABA(A) receptor-mediated currents in the amygdala: a mechanism for RDX-induced seizures. Environ Health Perspect, 119(3):357-363.

Appendix 1

List of MIEs in this AOP

Event: 667: Binding at picrotoxin site, iGABAR chloride channel

Short Name: Binding at picrotoxin site, iGABAR chloride channel

Key Event Component

Process	Object	Action
GABA receptor binding	gamma-aminobutyric acid receptor subunit alpha-1	increased
GABA receptor binding	gamma-aminobutyric acid receptor subunit alpha-5	increased
GABA receptor binding	gamma-aminobutyric acid receptor subunit alpha-6	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:10 - Binding to the picrotoxin site of ionotropic GABA receptors leading to epileptic seizures in adult brain	MolecularInitiatingEvent

Stressors

Name
Picrotoxin
Lindane
Fipronil
RDX
Alpha-endosulfan
Penicillin

Biological Context

Level of Biological Organization
Molecular

Cell term

Cell term
neuron

Organ term

Organ term
brain

Evidence for Perturbation by Stressor

Overview for Molecular Initiating Event

Chemicals non-competitively bind at or near the central pore of the receptor complex (e.g., the picrotoxin site) and directly block chloride conductance through the ion channel (Kalueff 2007). It has been postulated that they fit a single "big picrotoxin binding pocket" in the chloride channel lumen lined by five TM2 segments (Kalueff 2007; Olsen 2006). This hypothesis was examined with the β3 homopentamer by mutagenesis, pore structure studies, ligand binding, and molecular modeling (Chen et al. 2006). Results suggest that they fit the 2' to 9' pore region forming hydrogen bonds with the T6' hydroxyl and hydrophobic interactions with A2', T6', and L9' alkyl substituents, thereby blocking the channel. More computational evidence can be found in Sander et al. (2011), Carpenter et al. (2013) and Zheng et al. (2014).

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
mouse	Mus musculus	High	NCBI
dogs	Canis lupus familiaris	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Adult	High

Sex Applicability

Sex	Evidence
Unspecific	High

Theoretically, this MIE is applicable to any organisms that possess ionotropic GABA receptors (iGABARs) in their central and/or peripheral nervous systems. Many reviews (e.g., Hoisie *et al*. 1997; Buckingham *et al*. 2005; Michels and Moss 2007; Olsen and

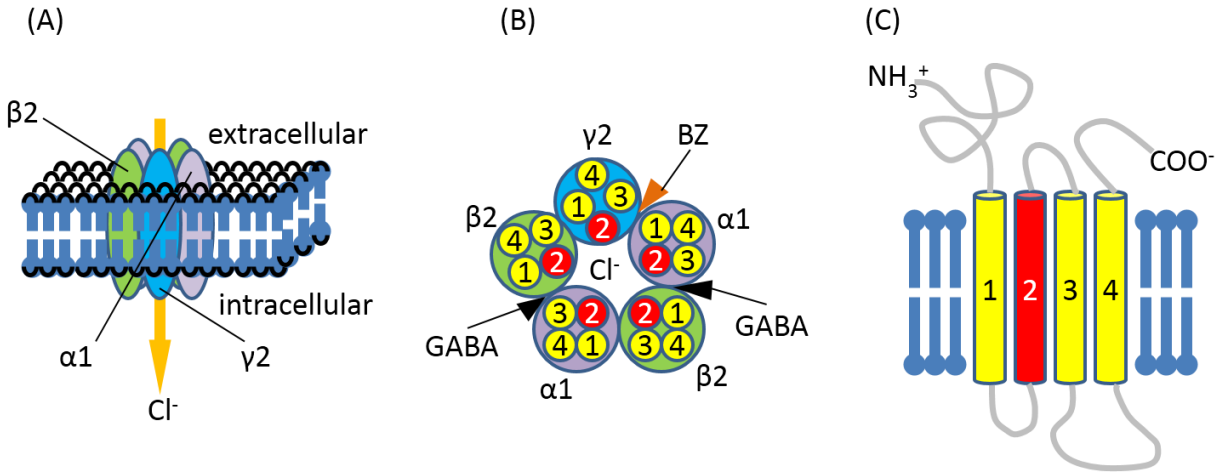
Sieghart 2009) have summarized evidence of ubiquitous existence of iGABARs ($GABA_A$-R in vertebrates including the humans) in species spanning from invertebrates to human. For instance, an ionotropic GABA receptor gene (GABA-receptor subunit-encoding Rdl gene) was isolated from a naturally occurring dieldrin-resistant strain of *D. melanogaster* (Ffrench-Constant *et al*., 1991,1993; Ffrench-Constant and Rocheleau, 1993). Nineteen $GABA_A$ receptor genes have been identified in the human genome (Simon *et al*. 2004). Direct evidence is mostly derived from *in silico* molecular modeling that docks ligands to the binding pockets of iGABARs in human (Carpenter *et al*. 2013; Chen *et al*. 2006; Sander *et al*. 2011), fruitfly and zebrafish (Zheng *et al*. 2014).

It should be noted that this MIE is limited to adult brains because of the age- or developmental stage-dependent nature of iGABAR where GABA acts as an excitatory (instead of inhibitory) neurotransmitter due to a higher intracellular chloride concentration in immature or developing neurons (Taketo and Yoshioka 2000; Galanopoulou 2008; Ben-Ari 2006).

This MIE is not applicable to $GABA_B$ receptors because, as metabotropic G-protein-coupled receptors, they mediate slow and sustained inhibitory responses and are involved in absence epilepsy (Han *et al*. 2012).

Key Event Description

Figure 1. Structure of ionotropic GABA receptors based on the consensus in multiple literature reviews (Source: Gong et al. 2015).



Shown is a common subtype α1β2γ2 of GABAA receptors found in the mammalian CNS. (A) Five subunits from three subunit subfamilies assemble to form a heteropentameric chloride permeable channel. (B) Stoichiometry and subunit arrangement of the GABAA receptor. Also shown are the binding sites for GABA and BZ. (C) Receptor subunits consist of four hydrophobic transmembrane domains (TM1-4), where TM2 is believed to line the pore of the channel. The large extracellular N-terminus is the site for ligand binding as well as the site of action of various drugs (e.g., picrotoxin and dieldrin). Each receptor subunit also contains a large intracellular domain between TM3 and TM4, which is the site for various protein–protein interactions as well as the site for post-translational modifications that modulate receptor activity. BZ: Benzodiazepines; CNS: Central nervous system; TM: Transmembrane.

As shown in Figure 1, binding of GABA opens up the chloride channel in the iGABAR complex, leading to neuronal inhibition (Olsen *et al*. 2004). Non-competitive channel blockers (e.g., fipronil, lindane, picrotoxin, penicillin and alpha-endosulfan) indirectly modulate the iGABAR activity (i.e., alter the response of the receptor to agonist) by noncompetitively binding at or near the central pore of the receptor complex (e.g., the picrotoxin site), an allosteric site distinct from that of the orthosteric agonist binding site, and inducing a conformational change within the receptor (Ernst *et al*. 2005; Johnston 2005).

Mounting evidence indicates that iGABAR chloride channel blockers bind to overlapping but not identical sites (Kalueff 2007). Based on the mapping of convulsants' binding domains to the iGABAR chloride ionophore (Olsen 2006), Kalueff (2007) proposed a "big picrotoxin binding pocket" model for the ligand-binding area of ionophore. For instance, bicyclophosphates, butyrolactones, pentylenetetrazole and penicillin act on the N-terminus of TM2 (‘‘main’’ picrotoxin binding site), whereas picrotoxin may act on the "main" as well as a hypothetical second ‘‘allosteric’’ picrotoxin site located on the C-terminus of TM2 (Kalueff 2007; Olsen 2006). In spite of minor differences, these blockers share similarity in channel blockage characteristics (e.g., voltage-dependency, binding to closed channel, reversible, and non-competitive) and effects of channel state (open/closed frequency and duration) (Kalueff 2007). Nevertheless, the 3D structure of iGABAR ionophore is unavailable. More homology modeling work (similar to the electron microscopic structure of nicotinic receptor channel (Unwin 2005)) may allow us to gain in-depth understanding of the picrotoxin site's accessibility for and interactions with channel blockers (i.e., convulsants), clarify the biological functions of the binding sites, and verify the impact of mutations in TM2 on ligand binding activity (Kalueff 2007).

How it is Measured or Detected

Binding to a specific site on iGABAR can be determined using a variety of methods including mutagenesis, pore structure studies, ligand binding, and molecular modeling (more details on methods can be found in Chen *et al*. 2006). One should choose a method in accordance with specific goal and also on the basis of available laboratory facilities. For example, Atack *et al*. (2007) chose the radioligand [^{35}S]*t*-butylbicyclophosphorothionate (TBPS) binding assay to determine the binding properties (i.e., inhibition by TBPS, picrotoxin, loreclezole and pentobarbital and modulation by GABA) at the convulsant binding site.

References

Atack JR, Ohashi Y, McKernan RM. 2007. Characterization of [^{35}S]t-butylbicyclophosphorothionate ([^{35}S]TBPS) binding to $GABA_A$ receptors in postmortem human brain. Br J Pharmacol. 150(8):1066-74.

Buckingham SD, Biggin PC, Sattelle BM, Brown LA, Sattelle DB. 2005. Insect GABA receptors: splicing, editing, and targeting by antiparasitics and insecticides. Mol Pharmacol 68(4):942-951.

Ben-Ari Y. 2006. Seizures beget seizures: the quest for GABA as a key player. Crit Rev Neurobiol 18(1-2): 135-44.

Carpenter TS, Lau EY, Lightstone FC. 2013. Identification of a possible secondary picrotoxin-binding site on the GABAA receptor. Chem Res Toxicol. 26(10):1444-54.

Chen L, Durkin KA, Casida JE. 2006. Structural model for gamma-aminobutyric acid receptor noncompetitive antagonist binding: widely diverse structures fit the same site. Proc Natl Acad Sci USA 103(13):5185-5190.

Ernst M, Bruckner S, Boresch S, Sieghart W. 2005. Comparative models of $GABA_A$ receptor extracellular and transmembrane domains: important insights in pharmacology and function. Mol Pharmacol 68(5):1291-1300.

Ffrench-Constant RH, Mortlock DP, Shaffer CD, MacIntyre RJ, Roush RT. 1991. Molecular cloning and transformation of cyclodiene resistance in Drosophila: an invertebrate gamma-aminobutyric acid subtype A receptor locus. Proc Natl Acad Sci USA 88:7209–7213.

Ffrench-Constant RH and Rocheleau TA. 1993 Drosophila gamma-aminobutyric acid receptor gene Rdl shows extensive alternative splicing. J Neurochem 60:2323–2326.

Ffrench-Constant RH, Steichen JC, Rocheleau TA, Aronstein K, and Roush RT. 1993. A single-amino acid substitution in a gamma-aminobutyric acid subtype A receptor locus is associated with cyclodiene insecticide resistance in Drosophila populations. Proc Natl Acad Sci USA 90:1957–1961.

Galanopoulou AS. 2008. $GABA_A$ Receptors in Normal Development and Seizures: Friends or Foes? Curr Neuropharmacol. 6(1):1-20.

Gong P, Hong H, Perkins EJ. 2015. Ionotropic GABA receptor antagonism-induced adverse outcome pathways for potential neurotoxicity biomarkers. Biomarkers in Medicine 9(11):1225-39.

Han HA, Cortez MA, Snead OC III. 2012. $GABA_B$ Receptor and Absence Epilepsy. In: Noebels JL, Avoli M, Rogawski MA, et al., editors. Jasper's Basic Mechanisms of the Epilepsies [Internet]. 4th edition. Bethesda (MD): National Center for Biotechnology Information (US); 2012. Available from: https://www.ncbi.nlm.nih.gov/books/NBK98192/.

Hosie AM, Aronstein K, Sattelle DB, Ffrench-Constant RH. 1997. Molecular biology of insect neuronal GABA receptors. Trends Neurosci 20(12):578-583.

Johnston GA. 2005. GABA(A) receptor channel pharmacology. Curr Pharm Des 11(15):1867-1885.

Kalueff AV. 2007. Mapping convulsants' binding to the GABA-A receptor chloride ionophore: a proposed model for channel binding sites. Neurochem Int 50(1): 61-68.

Michels G, Moss SJ. 2007. GABAA receptors: properties and trafficking. Crit Rev Biochem Mol Biol 42(1):3-14.

Olsen RW. 2006. Picrotoxin-like channel blockers of $GABA_A$ receptors. Proc Natl Acad Sci USA. 103:6081-82.

Olsen RW, Chang C-SS, Li G, Hanchar HJ, Wallner M. 2004. Fishing for allosteric sites on GABAa receptors. Biochem Pharmacol. 68:1675-84.

Olsen RW, Sieghart W. 2009. $GABA_A$ receptors: subtypes provide diversity of function and pharmacology. Neuropharmacology. 56(1):141-8.

Sander T, Frolund B, Bruun AT, Ivanov I, McCammon JA, Balle T. 2011. New insights into the GABAA receptor structure and orthosteric ligand binding: receptor modeling guided by experimental data. Proteins. 79(5):1458-77.

Simon J, Wakimoto H, Fujita N, Lalande M, Barnard EA. 2004. Analysis of the set of GABA(A) receptor genes in the human genome. J. Biol. Chem. 279(40), 41422–41435.

Taketo M, Yoshioka T. 2000. Developmental change of $GABA_A$ receptor-mediated current in rat hippocampus. Neuroscience 96(3):507-514.

Unwin N. 2005. Refined structure of the nicotinic acetylcholine receptor at 4 A˚ resolution. J Mol Biol. 346:967-89.

Zheng N, Cheng J, Zhang W, Li W, Shao X, Xu Z, Xu X, Li Z. 2014. Binding difference of fipronil with GABAARs in fruitfly and zebrafish: insights from homology modeling, docking, and molecular dynamics simulation studies. J Agric Food Chem 62(44):10646-53.

List of Key Events in the AOP

Event: 64: Reduction, Ionotropic GABA receptor chloride channel conductance

Short Name: Reduction, Ionotropic GABA receptor chloride channel conductance

Key Event Component

Process	Object	Action
GABA-gated chloride ion channel activity	chloride	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:10 - Binding to the picrotoxin site of ionotropic GABA receptors leading to epileptic seizures in adult brain	KeyEvent

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
neuron

Organ term

Organ term
brain

Domain of Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI
rats	Rattus norvegicus	High	NCBI
mouse	Mus musculus	High	NCBI
Drosophila melanogaster	Drosophila melanogaster	High	NCBI

Banerjee et al. (1999) reported functional modulation of GABAA receptors by Zn2+, pentobarbital, neuroactive steroid alphaxalone, and flunitrazepam in the cerebral cortex and cerebellum of rats undergoing status epilepticus induced by pilocarpine.

Babot et al. (2007) measured the reduction in mouse GABAA receptor function by 3 μM dieldrin using the GABA-induced 36Cl- uptake method.

Bromfield et al. (2006) reviewed evidence for GABAA receptors in human and mammalian brains, whereas Narahashi (1996) and Costa (2015) reviewed organochlorine and some pyrethroid compounds as insecticides with the target site of chloride channel.

Grolleau and Sattelle (2000) reported a complete blocking of inward current by 100 μM picrotoxin in the wild-type RDL (iGABAR) of Drosophila melanogaster.

Key Event Description

This key event occurs at the cellular level and is characterized by a dose-dependent post-synaptic inhibition of membrane currents in iGABAR-containing cells, especially neuronal cells (Dichter and Ayala 1987; Bromfield et al. 2006), leading to the reduction of iGABAR chloride channel conductance.

How it is Measured or Detected

The change in membrane conductance can be measured by determining the alteration (i.e., inhibition) in muscimol-stimulated (Banerjee et al. 1999) or GABA-induced uptake (Babot et al. 2007) of $^{36}Cl^-$ in cortical and cerebellar membranes or primary cerebellar granule cell cultures, prior to and after exposure to a GABA antagonist. Inglefield and Schwartz-Bloom (1998) reported a Cl^--sensitive fluorescent dye-based method where to measure real-time changes in intracellular chloride concentration with UV laser scanning confocal microscopy.

References

Babot Z, Vilaro MT, Sunol C. (2007) Long-term exposure to dieldrin reduces gamma-aminobutyric acid type A and N-methyl-D-aspartate receptor function in primary cultures of mouse cerebellar granule cells. J. Neurosci. Res. 85(16), 3687-3695.

Banerjee PK, Olsen RW, Snead OC, III. (1999) Zinc inhibition of gamma-aminobutyric acid(A) receptor function is decreased in the cerebral cortex during pilocarpine-induced status epilepticus. J Pharmacol Exp Ther 1999; 291(1):361-366.

Bromfield EB, Cavazos JE, Sirven JI. (2006) Chapter 1, Basic Mechanisms Underlying Seizures and Epilepsy. In: An Introduction to Epilepsy [Internet]. West Hartford (CT): American Epilepsy Society; Available from: http://www.ncbi.nlm.nih.gov/books/NBK2510

Costa LG. (2015) The neurotoxicity of organochlorine and pyrethroid pesticides. Handb Clin Neurol. 131:135-48.

Dichter MA, Ayala GF. (1987) Cellular mechanisms of epilepsy: a status report. Science 237(4811), 157-164.

Gong P. Hong HH, Perkins EJ. (2015) Ionotropic GABA receptor antagonism-induced adverse outcome pathways for potential neurotoxicity biomarkers. Biomark. Med. 9(11):1225-39.

Grolleau F, Sattelle DB. (2000) Single channel analysis of the blocking actions of BIDN and fipronil on a Drosophila melanogaster GABA receptor (RDL) stably expressed in a Drosophila cell line. Br J Pharmacol. 130(8):1833-42.

Inglefield JR, Schwartz-Bloom RD. (1998) Optical imaging of hippocampal neurons with a chloride-sensitive dye: early effects of in vitro ischemia. J Neurochem. 70(6):2500-9.

Narahashi T. (1996). Neuronal ion channels as the target sites of insecticides. Pharmacol Toxicol. 79(1):1-14.

Event: 669: Reduction, Neuronal synaptic inhibition

Short Name: Reduction, Neuronal synaptic inhibition

Key Event Component

Process	Object	Action
chemical synaptic transmission		decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:10 - Binding to the picrotoxin site of ionotropic GABA receptors leading to epileptic seizures in adult brain	KeyEvent

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
neuron

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	High	NCBI
guinea pig	Cavia porcellus	High	NCBI
human	Homo sapiens	High	NCBI
Japanese quail	Coturnix japonica	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Adult	High

Sex Applicability

Sex	Evidence
Unspecific	High

See Juarez et al. (2013) for supporting evidence for Guinea pig. For rat, whole-cell in vitro recordings in the rat basolateral amygdala (BLA) showed that RDX reduces the frequency and amplitude of GABAA receptor mediated sIPSCs and the amplitude of GABA-evoked postsynaptic currents, whereas in extracellular field recordings from the BLA, RDX induced prolonged, seizure-like neuronal discharges (Williams et al, 2011).

Key Event Description

A reduction in GABA-mediated inhibition of neuronal synaptic signaling is reflected as decreased frequency and amplitude of iGABAR-mediated spontaneous inhibitory postsynaptic currents (sIPSCs) or abolishment of GABA-induced firing action (Newland and Cull-Candy 1992).

How it is Measured or Detected

Juarez et al. (2013) used primary cultured neurons obtained from the guinea-pig small intestine to detect picrotoxin concentration-dependent (and reversible) inhibition of GABA-induced membrane currents. Williams et al. (2011) used whole-cell in vitro recordings in the rat basolateral amygdala (BLA) to detect the reduced frequency and amplitude of $GABA_A$ receptor mediated spontaneous inhibitory postsynaptic currents (sIPSCs) and the amplitude of GABA-evoked postsynaptic currents, both of which were induced by RDX.

References

Newland C F, Cull-Candy S G. On the mechanism of action of picrotoxin on GABA receptor channels in dissociated sympathetic neurones of the rat. J Physiol 1992; 447: 191–213.

Juarez E H, Ochoa-Cortes F, Miranda-Morales M, Espinosa-Luna R, Montano L M, Barajas-Lopez C. Selectivity of antagonists for the Cys-loop native receptors for ACh, 5-HT and GABA in guinea-pig myenteric neurons. Auton Autacoid Pharmacol 2013; 34(1-2):1-8.

Williams L R, Aroniadou-Anderjaska V, Qashu F, Finne H, Pidoplichko V, Bannon D I et al. RDX binds to the GABA(A) receptor-convulsant site and blocks GABA(A) receptor-mediated currents in the amygdala: a mechanism for RDX-induced seizures. Environ Health Perspect 2011; 119(3):357-363.

Event: 682: Generation, Amplified excitatory postsynaptic potential (EPSP)

Short Name: Generation, Amplified excitatory postsynaptic potential (EPSP)

Key Event Component

Process	Object	Action
excitatory postsynaptic potential		occurrence

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:10 - Binding to the picrotoxin site of ionotropic GABA receptors leading to epileptic seizures in adult brain	KeyEvent

Biological Context

Level of Biological Organization
Cellular

Organ term

Organ term
brain

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	High	NCBI
rat	Rattus norvegicus	High	NCBI
guinea pig	Cavia porcellus	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Adult	High

Sex Applicability

Sex	Evidence
Unspecific	High

Miura et al. (1997) reported supporting evidence from guinea pigs whereas Dichter and Ayala (1987) and Bromfield et al. (2006) summarized relevant studies on humans. Acker et al. (2016) perform simultaneous two-photon voltage-sensitive dye recording with two-photon glutamate uncaging in order to measure the characteristics (amplitude and duration) of uncaging-evoked EPSPs in acute mouse brain slices.

Key Event Description

In neuroscience, an excitatory postsynaptic potential (EPSP) is defined as a neurotransmitter-induced postsynaptic potential change that depolarizes the cell, and hence increases the likelihood of initiating a postsynaptic action potential (Purves et al. 2001). On the contrary, an inhibitory postsynaptic potential (IPSP) decreases this likelihood. Whether a postsynaptic response is an EPSP or an IPSP depends on the type of channel that is coupled to the receptor, and on the concentration of permeant ions inside and outside the cell. In fact, the only factor that distinguishes postsynaptic excitation from inhibition is the reversal potential of the postsynaptic potential (PSP) in relation to the threshold voltage for generating action potentials in the postsynaptic cell. When an active presynaptic cell releases neurotransmitters into the synapse, some of them bind to receptors on the postsynaptic cell. Many of these receptors contain an ion channel capable of passing positively charged ions (e.g., Na^+ or K^+) or negatively charged ions (e.g., Cl^-) either into or out of the cell. In epileptogenesis, discharges reduced $GABA_A$ receptor-mediated hyperpolarizing IPSPs by shifting their reversal potentials in a positive direction. At the same time, the amplitudes of Schaffer collateral-evoked RS-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor-mediated EPSPs and action potential-independent miniature EPSPs were enhanced, whereas N-methyl-d-aspartate receptor-mediated EPSPs remained unchanged. Together, these changes in synaptic transmission produce a sustained increase in hippocampal excitability (Lopantsev et al. 2009).

How it is Measured or Detected

EPSPs are usually recorded by measuring electrical responses and changes in intracellular calcium concentration using intracellular electrodes (Miura et al. 1997) or recording extracellular electrical activity or potential using >20 electroencephalogram (EEG) electrodes (often in clinical settings) (Bromfield et al. 2006). Recently, voltage-sensitive dyes have been successfully used for measuring voltage responses from large neuronal populations in acute brain slice preparations (Popovic et al. 2015; Acker et al. 2016).

References

Acker CD, Hoyos E, Loew LM. (2016) EPSPs Measured in Proximal Dendritic Spines of Cortical Pyramidal Neurons. eNeuro. 3(2) ENEURO.0050-15.2016.

Bromfield EB, Cavazos JE, Sirven JI. (2006) Chapter 1, Basic Mechanisms Underlying Seizures and Epilepsy. In: An Introduction to Epilepsy [Internet]. West Hartford (CT): American Epilepsy Society; Available from: http://www.ncbi.nlm.nih.gov/books/NBK2510/.

Dichter MA, Ayala GF. (1987) Cellular mechanisms of epilepsy: A status report. Science 237:157-64.

Lopantsev V, Both M, Draguhn A. 2009. Rapid Plasticity at Inhibitory and Excitatory Synapses in the Hippocampus Induced by Ictal Epileptiform Discharges. Eur J Neurosci 29(6):1153–64.

Miura M, Yoshioka M, Miyakawa H, Kato H, Ito KI. (1997) Properties of calcium spikes revealed during $GABA_A$ receptor antagonism in hippocampal CA1 neurons from guinea pigs. J Neurophysiol. 78(5):2269-79.

Popovic MA, Carnevale N, Rozsa B, Zecevic D. (2015) Electrical behaviour of dendritic spines as revealed by voltage imaging. Nature Communications. 6:8436.

Purves D, Augustine GJ, Fitzpatrick D, Katz LC, LaMantia A-S, McNamara JO, Williams SM (Eds). 2001. Neuroscience. 2nd edition. Chapter 7. Neurotransmitter Receptors and Their Effects. Sunderland (MA): Sinauer Associates. Available from: http://www.ncbi.nlm.nih.gov/books/NBK10799/.

Event: 616: Occurrence, A paroxysmal depolarizing shift

Short Name: Occurrence, A paroxysmal depolarizing shift

Key Event Component

Process	Object	Action
membrane depolarization		occurrence

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:10 - Binding to the picrotoxin site of ionotropic GABA receptors leading to epileptic seizures in adult brain	KeyEvent

Biological Context

Level of Biological Organization
Tissue

Organ term

Organ term
brain

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI
rat	Rattus norvegicus	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Adult	High

Sex Applicability

Sex	Evidence
Unspecific	High

Most of the supporting evidence comes from studies on human and rodents. See the reviews of Bromfield (2006) and Lomen-Hoerth and Messing (2010) for examples.

Key Event Description

A paroxysmal depolarizing shift (PDS) or depolarizing shift is a cellular manifestation of epilepsy. As summarized by Lomen-Hoerth and Messing (2010), brain electrical activity is nonsynchronous under normal conditions. In epileptic seizures, a large group of neurons begin firing in an abnormal, excessive, and synchronized manner, which results in a wave of depolarization known as a paroxysmal depolarizing shift (Somjen, 2004). Normally after an excitatory neuron fires it becomes more resistant to firing for a period of time, owing in part to the effect of inhibitory neurons, electrical changes within the excitatory neuron, and the negative effects of adenosine. However, in epilepsy the resistance of excitatory neurons to fire during this period is decreased, likely due to changes in ion channels or inhibitory neurons not functioning properly. This then results in a specific area from which seizures may develop, known as a "seizure focus".

Increased, abnormal neuron firing causes a wave of depolarization throughout the brain/neuronal tissue. At the level of single neurons, epileptiform activity consists of sustained neuronal depolarization resulting in a burst of action potentials, a plateau-like depolarization associated with completion of the action potential burst, and then a rapid repolarization followed by hyperpolarization. This sequence is also called paroxysmal depolarizing shift (PDS). The bursting activity resulting from the relatively prolonged depolarization of the neuronal membrane is due to influx of extracellular Ca^{2+}, which leads to the opening of voltage-dependent Na^{+} channels, influx of Na^{+}, and generation of repetitive action potentials. The subsequent hyperpolarizing afterpotential is mediated by iGABA receptors and Cl^{-} influx, or by K^{+} efflux, depending on the cell type (Bromfield *et al*. 2006).

How it is Measured or Detected

Paroxysmal depolarizing shifts can be measured *in vitro* using patch-clamp recording technique (Kapur 2009) or micro-electrode arrays (Novellino *et al*. 2011) to determine effects of chemicals on action potential patterns of neurons.

PDS can be detected *in vivo* using electroencephalography techniques (Niedermeyer and da Silva 2005).

References

Bromfield EB, Cavazos JE, Sirven JI. 2006. An Introduction to Epilepsy [Internet]. West Hartford (CT): American Epilepsy Society; Chapter 1, Basic Mechanisms Underlying Seizures and Epilepsy. Available from: http://www.ncbi.nlm.nih.gov/books/NBK2510/.

Kapur J. 2009. GABA | Pathophysiology of Status Epilepticus. Encyclopedia of Basic Epilepsy Research, pp 304-8.

Lomen-Hoerth C, Messing RO. 2010. Chapter 7: Nervous system disorders. Edited by Stephen J. McPhee, and Gary D. Hammer, Pathophysiology of disease: an introduction to clinical medicine (6th Edition). New York: McGraw-Hill Medical. ISBN 9780071621670.

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.

Niedermeyer E, da Silva FL. 2005. Electroencephalography: basic principles, clinical applications, and related fields. Lippincott Williams & Wilkins.

Somjen GG. 2004. Ions in the Brain Normal Function, Seizures, and Stroke. New York: Oxford University Press. p. 167.

List of Adverse Outcomes in this AOP

Event: 613: Occurrence, Epileptic seizure

Short Name: Occurrence, Epileptic seizure

Key Event Component

Process	Object	Action
seizures		occurrence

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:10 - Binding to the picrotoxin site of ionotropic GABA receptors leading to epileptic seizures in adult brain	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
mouse	Mus musculus	High	NCBI
honeybee	Apis mellifera	High	NCBI
eisenia fetida	eisenia fetida	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Adult	High

Sex Applicability

Sex	Evidence
Unspecific	High

Substance-induced epileptic seizures have been documented in a wide range of species including invertebrates and vertebrates (see Tingle *et al*. (2003) and Gunasekara *et al*. (2007) for reviews on the list of aquatic and terrestrial species affected by fipronil). For instance, fipronil can induce seizures in fruit flies (Stilwell *et al*. 2006) and house flies (Gao *et al*. 2007).

Key Event Description

Blockage of the GABA-gated chloride channel reduces neuronal inhibition and induces focal seizure. This may further lead to generalized seizure, convulsions and death (Bloomquist 2003; De Deyn *et al*. 1990; Werner and Covenas 2011). For instance, exposure to fipronil produces hyperexcitation at low doses and convulsion or tonic-clonic seizure and seizure-related death at high doses (Gunasekara *et al*. 2007; Tingle *et al*. 2003; Jackson *et al*. 2009).

As described in Bromfield *et al*. (2006), seizure propagation, the process by which a partial seizure spreads within the brain, occurs when there is sufficient activation to recruit surrounding neurons. This leads to a loss of surround inhibition and spread of seizure activity into contiguous areas via local cortical connections, and to more distant areas via long association pathways such as the corpus callosum. The propagation of bursting activity is normally prevented by intact hyperpolarization and a region of surrounding inhibition created by inhibitory neurons. With sufficient activation there is a recruitment of surrounding neurons via a number of mechanisms. Of equal interest, but less well understood, is the process by which seizures typically end, usually after seconds or minutes, and what underlies the failure of this spontaneous seizure termination in the life-threatening condition known as status epilepticus (Bromfield *et al*. 2006).

How it is Measured or Detected

Electrophysiological measurements and physical (visual) observation (for mortality) are the methods often used to detect epileptic seizure-related effects (Ulate-Campos *et al*. 2016). One may also visit http://www.mayoclinic.org/diseases-conditions/epilepsy/diagnosis-treatment/diagnosis/dxc-20117234 for more information on how medical doctors diagnose epilepsy in patients.

Recently, a new technique called micro-electrode array (MEA) recording has been developed and tested both *in vitro* (Novellino *et al*. 2011) and *ex vivo* (Dossi *et al*. 2014). MEAs, which are microfabricated devices embedding an array of spatially arranged microelectrodes, provide a unique opportunity to simultaneously stimulate and record field potentials, as well as action potentials of multiple neurons from different areas of the tissue (Dossi *et al*. 2014). Thus, MEAs recordings constitute an excellent tool for studying the spatio-temporal patterns of spontaneous interictal and evoked seizure-like events, the mechanisms underlying seizure onset and propagation, and electrophysiological activity of the neurons in response to chemical exposures (Novellino *et al*. 2011; Dossi *et al*. 2014).

Regulatory Significance of the AO

As a neurotoxicity endpoint, information with regard to the seizure or epilepsy is often used by regulators such as EPA, FDA and DHS for human and environmental health assessment and regulation of chemicals, drugs and other materials. For instance, the Office of Pesticide Programs (OPP) in US EPA, regulates, monitors and investigates the use of all pesticides in accordance with the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (https://www.epa.gov/laws-regulations/summary-federal-insecticide-fungicide-and-rodenticide-act). Many pesticides like fipronil target the iGABAR causing seizure and mortality. Another example is the regulatory actions of US FDA to ensure drug safety (see https://www.fda.gov/Drugs/DrugSafety/ucm436494.htm).

References

Bloomquist JR. 2003. Chloride channels as tools for developing selective insecticides. Arch. Insect Biochem. Physiol 54(4), 145-156.

Bromfield EB, Cavazos JE, Sirven JI, editors. 2006. An Introduction to Epilepsy [Internet]. West Hartford (CT): American Epilepsy Society. Chapter 1 Basic Mechanisms Underlying Seizures and Epilepsy. Available from: http://www.ncbi.nlm.nih.gov/books/NBK2510/

De Deyn PP, Marescau B, Macdonald RL. 1990. Epilepsy and the GABA-hypothesis a brief review and some examples. Acta Neurol. Belg. 90(2), 65-81.

Dossi E, Blauwblomme T, Nabbout R, Huberfeld G, Rouach N. 2014. Multi-electrode array recordings of human epileptic postoperative cortical tissue.J Vis Exp. (92):e51870.

Gao JR, Kozaki T, Leichter CA, Rinkevich FD, Shono T, Scott JG. 2007. The A302S mutation in Rdl that confers resistance to cyclodienes and limited crossresistance to fipronil is undetectable in field populations of house flies from the USA. Pestic. Biochem. Physiol. 88, 66−70.

Gunasekara AS, Truong T, Goh KS, Spurlock F, Tjeerdema RS. 2007. Environmental fate and toxicology of fipronil. J. Pestic. Sci. 32(3), 189-199.

Jackson D, Cornell CB, Luukinen B, Buhl K, Stone D. 2009. Fipronil Technical Fact Sheet. National Pesticide Information Center, Oregon State University Extension Services,

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.

Stilwell GE, Saraswati S, J. Troy Littleton JT, Chouinard SW. 2006. Development of a Drosophila seizure model for in vivo high-throughput drug screening. European J Neurosci. 24, 2211-2222.

Tingle CC, Rother JA, Dewhurst CF, Lauer S, King WJ. 2003. Fipronil: environmental fate, ecotoxicology, and human health concerns. Rev. Environ. Contam Toxicol. 176, 1-66.

Ulate-Campos A, Coughlin F, Gaínza-Lein M, Fernández IS, Pearl PL, Loddenkemper T. 2016. Automated seizure detection systems and their effectiveness for each type of seizure. Seizure. 40:88-101.

Werner FM, Covenas R. 2011. Classical neurotransmitters and neuropeptides involved in generalized epilepsy: a focus on antiepileptic drugs. Curr. Med. Chem. 18(32), 4933-4948.

Appendix 2

List of Adjacent Key Event Relationships

Relationship: 666: Binding at picrotoxin site, iGABAR chloride channel leads to Reduction, Ionotropic GABA receptor chloride channel conductance

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Binding to the picrotoxin site of ionotropic GABA receptors leading to epileptic seizures in adult brain	adjacent	High	High

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI
zebrafish	Danio rerio	High	NCBI
rat	Rattus norvegicus	High	NCBI
Drosophila melanogaster	Drosophila melanogaster	High	NCBI

Due to the universal existence of iGABARs in the animal kingdom, it would be a very long list of studies that provide supporting evidence with regard to taxonomic applicability of this key event relationship. The following are two examples: Williams et al. (2011) determined the binding affinity of RDX to the picrotoxin-binding site and the blockage of GABA(A) receptor-mediated currents in the rat amygdala; Grolleau and Sattelle (2000) reported a complete blocking of inward current by 100 μM picrotoxin in the wild-type RDL (iGABAR) of *Drosophila melanogaster*.

Key Event Relationship Description

Acting as the major inhibitory neurotransmitter receptors, the ionotropic GABA receptors (iGABARs) are ligand-gated ion channels (LGICs) (Carpenter et al. 2013). Upon binding of an agonist (e.g., GABA), the iGABAR opens and increases the intraneuronal concentration of chloride ions, thus hyperpolarizing the cell and inhibiting the transmission

of the nerve action potential. iGABARs also contain many other modulatory binding pockets that differ from the agonist-binding site. The picrotoxin-binding site is a noncompetitive channel blocker site located at the cytoplasmic end of the transmembrane channel (Olsen 2015). Binding to this pocket blocks GABA-induced chloride current, hence reduces chloride conductance.

Evidence Supporting this KER

Biological Plausibility

The mechanisms for noncompetitive picrotoxin site binding-induced reduction in chloride conductance have been investigated intensively for several decades. The consensus has been reached with ample support of computational and experimental evidence. Noncompetitive channel blockers fit the 2' to 9' pore region forming hydrogen bonds with the T6' hydroxyl and hydrophobic interactions with A2', T6' and L9' alkyl substituents (Chen et al. 2006), which is the primary binding site in the chloride channel lumen lined by five TM2 segments, thereby blocking the channel. Recent evidence suggests that there also exists a secondary modulatory pocket at the interface between the ligand-binding domain and the transmembrane domain of the iGABAR (Carpenter et al. 2013). It is believed that the two mechanisms mediate the blockage of chloride conductance (Yoon et al. 1993; Carpenter et al. 2013).

Empirical Evidence

Numerous pharmacological and computational studies have lent strong support for this relationship. For instance, picrotoxin, applied intracellularly, was capable of blocking GABA-activated chloride current (Akaike et al. 1985). Recently, a computational study using homology modeling, docking and molecular dynamics simulation methods revealed that difference in binding affinity of fipronil with different iGABARs may lead to differential toxicity (potency) (Zheng et al. 2014).

Uncertainties and Inconsistencies

As a heteropentameric receptor, the iGABAR consists of five protein subunits arranged around a central pore that form an ion channel through the membrane. The subunits are drawn from a pool of 19 distinct gene products, including six alpha, three beta, and three gamma subunits. The high diversity of subunit genes, in combination with alternative splicing and editing, leads to an enormous variety and, consequently, variability in function and sensitivity. This constitutes the main source of uncertainties.

References

Akaike N. Hattori K, Oomura Y, Carpenter D0. 1985. Bicuculline and picrotoxin block γ-aminobutyric acid-gated Cl-conductance by different mechanisms. Experientia 41:70-71.

Carpenter TS, Lau EY, Lightstone FC. 2013. Identification of a possible secondary picrotoxin-binding site on the GABA(A) receptor. Chem Res Toxicol. 26(10):1444-54.

Chen L, Durkin KA, Casida J. 2006. Structural model for gamma-aminobutyric acid receptor noncompetitive antagonist binding: widely diverse structures fit the same site. Proc Natl Acad Sci USA, 103(13):5185-5190.

Grolleau F, Sattelle DB. 2000. Single channel analysis of the blocking actions of BIDN and fipronil on a *Drosophila melanogaster* GABA receptor (RDL) stably expressed in a Drosophila cell line. Br J Pharmacol. 130(8):1833-42.

Olsen RW. 2015. Allosteric ligands and their binding sites define γ-aminobutyric acid (GABA) type A receptor subtypes. Adv Pharmacol. 73:167-202.

Williams LR, Aroniadou-Anderjaska V, Qashu F, Finne H, Pidoplichko V, Bannon D I et al. 2011. RDX binds to the GABA(A) receptor-convulsant site and blocks GABA(A) receptor-mediated currents in the amygdala: a mechanism for RDX-induced seizures. Environ Health Perspect. 119(3):357-363.

Yoon KW, Covey DF, Rothman SM. 1993. Multiple mechanisms of picrotoxin block of GABA-induced currents in rat hippocampal neurons. J Physiol. 464:423-39.

Zheng N, Cheng J, Zhang W, Li W, Shao X, Xu Z, Xu X, Li Z. 2014. Binding difference of fipronil with GABA(A)Rs in fruitfly and zebrafish: Insights from homology modeling, docking, and molecular dynamics simulation studies. J Agric Food Chem. 62:10646-53.

Relationship: 667: Reduction, Ionotropic GABA receptor chloride channel conductance leads to Reduction, Neuronal synaptic inhibition

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Binding to the picrotoxin site of ionotropic GABA receptors leading to epileptic seizures in adult brain	adjacent	High	High

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	High	NCBI
guinea pig	Cavia porcellus	High	NCBI

iGABARs and synaptic neurons are present in animals throughout the animal kingdom, therefore this event is applicable to a wide range of species from earthworm to humans. This relationship has been shown directly in rats (Williams et al. 2011) and guinea pig (Juarez et al. 2013).

Key Event Relationship Description

A decline in conductance through chloride channels in iGABARs causes a reduction in GABA-mediated inhibition of neuronal synaptic signaling, which is reflected as decreased frequency and amplitude of iGABAR-mediated spontaneous inhibitory postsynaptic currents or abolishment of GABA-induced firing action (Newland and Cull-Candy 1992). For instance, whole-cell *in vitro* recordings in the rat basolateral amygdala (BLA) showed that RDX reduces the frequency and amplitude of $GABA_A$ receptor mediated spontaneous inhibitory postsynaptic currents (sIPSCs) and the amplitude of GABA-evoked postsynaptic currents, whereas in extracellular field recordings from the BLA, RDX induced prolonged, seizure-like neuronal discharges (Williams et al. 2011). These pieces of cellular level evidence support that binding to the $GABA_A$ receptor convulsant site is the primary mechanism of seizure induction by RDX and that the key event of reduction of GABAergic

inhibitory transmission in the amygdala is involved in the generation of RDX-induced seizures.

Evidence Supporting this KER

Biological Plausibility

Chloride channels play an important role in regulating neuronal excitability, especially in the context of fast synaptic inhibition mediated by $GABA_A$ receptors. But in order for chloride channels to reduce excitability, chloride driving force must be maintained to keep a dynamic balancing of chloride influx and efflux, which also involves a variety of other ion species (Prescott 2014). If chloride regulation is compromised, the efficacy of fast synaptic inhibition can be compromised with adverse effects such as reduced neuronal inhibition.

Empirical Evidence

The $GABA_A$ receptor is part of a larger GABA/drug receptor-Cl^- ion channel macromolecular complex. An integral part of this complex is the Cl^- channel. The binding sites localized in or near the Cl^- channel for GABA, benzodiazepines, barbiturates, picrotoxin and anesthetic steroids modulate receptor response to GABA stimulation. The GABA-binding site is directly responsible for opening the Cl^- channel. Electrophysiological studies of the $GABA_A$-receptor complex indicate that it mediates an increase in membrane conductance with an equilibrium potential near the resting level of −70 mV. This conductance increase is often accompanied by a membrane hyperpolarization, resulting in an increase in the firing threshold and, consequently, a reduction in the probability of action potential initiation, causing neuronal inhibition (Olsen and DeLorey 1999). This reduction in membrane resistance is accomplished by the GABA-dependent facilitation of Cl^- ion influx through a receptor-associated channel.

Channel blockers, such as the convulsant compound picrotoxin, cause a decrease in mean channel open time. Picrotoxin acts on the gating process of the $GABA_A$ receptor channel and works by preferentially shifting opening channels to the briefest open state (1 msec). Experimental convulsants like pentylenetetrazol and the cage convulsant t-butyl bicyclophosphorothionate (TBPS) act in a manner similar to picrotoxin, preventing Cl^- channel permeability (Macdonald and Olsen 1994).

Uncertainties and Inconsistencies

As a heteropentameric receptor, the iGABAR consists of five protein subunits arranged around a central pore to form an ion channel through the membrane. The subunits are drawn from a pool of 19 distinct gene products, including six alpha, three beta, and three gamma subunits. The high diversity of subunit genes, in combination with alternative splicing and editing, leads to an enormous variety and, consequently, variability in function and sensitivity. This constitutes the main source of uncertainties.

References

Juarez E H, Ochoa-Cortes F, Miranda-Morales M, Espinosa-Luna R, Montano L M, Barajas-Lopez C. Selectivity of antagonists for the Cys-loop native receptors for ACh, 5-HT and GABA in guinea-pig myenteric neurons. Auton Autacoid Pharmacol 2013; 34(1-2):1-8.

Macdonald R L, Olsen R W. GABAA receptor channels. Annu. Rev. Neurosci. 1994;17:569–602.

Newland C F, Cull-Candy S G. On the mechanism of action of picrotoxin on GABA receptor channels in dissociated sympathetic neurones of the rat. J Physiol 1992; 447: 191–213.

Olsen R W, DeLorey T M. Chapter 16. GABA and Glycine: GABA Receptor Physiology and Pharmacology. In: Siegel GJ, Agranoff BW, Albers RW, et al. (Eds), Basic Neurochemistry: Molecular, Cellular and Medical Aspects (6th edition), Philadelphia: Lippincott-Raven; 1999.

Prescott S A. Chloride channels. In: Jaeger D and Jung R (Eds.), Encyclopedia of Computational Neuroscience, Springer, New York, 2014. pp.1-4.

Williams L R, Aroniadou-Anderjaska V, Qashu F, Finne H, Pidoplichko V, Bannon D I et al. RDX binds to the GABA(A) receptor-convulsant site and blocks GABA(A) receptor-mediated currents in the amygdala: a mechanism for RDX-induced seizures. Environ Health Perspect 2011; 119(3):357-363.

Relationship: 683: Reduction, Neuronal synaptic inhibition leads to Generation, Amplified excitatory postsynaptic potential (EPSP)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Binding to the picrotoxin site of ionotropic GABA receptors leading to epileptic seizures in adult brain	adjacent	High	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	High	NCBI
human	Homo sapiens	High	NCBI

El-Hassar et al. (2007) and Li et al. (1999) provided evidence for rat whereas Bartolomei et al. (2008) reported evidence for human.

Key Event Relationship Description

$GABA_A$ receptors mediate two distinct forms of inhibition, phasic and tonic. The first consists of fast inhibitory postsynaptic potentials (IPSPs), regulating point-to-point communication between neurons. The second consists of a persistent inhibitory conductance that plays a crucial role in regulating the membrane potential and network excitability (Farrant and Nusser 2005). In the case of phasic inhibition, synaptic $GABA_A$ receptors, facing presynaptic release sites, are activated by a brief exposure to a high concentration of GABA released by exocytosis of presynaptic vesicles. Once released, GABA diffuses throughout the neuropil before being taken up by selective plasma membrane transporters, which contribute to the clearance of the neurotransmitter (Cherubini and Conti 2001). In the case of tonic inhibition, extrasynaptic $GABA_A$ receptors, localized away from the synapses, are persistently exposed to low concentration of “ambient” GABA.

Fast inhibitory neurotransmission in the mammalian central nervous system (CNS) is mediated primarily by the neurotransmitters GABA and glycine. Glycine is predominantly used in the spinal cord and the brain stem, whereas GABA is more commonly used in the

brain (Jentsch et al. 2002). As the dominant charge carrier through GABA-A receptors, chloride is directly implicated in the efficacy of fast neuronal synaptic inhibition (Prescott 2014). The binding of GABA to $GABA_A$ receptors opens intrinsic anion channels, which leads to a Cl^- influx that hyperpolarizes the neuron and thereby inhibits postsynaptic neuronal activity in the adult CNS (Jentsch et al. 2002). Neurons communicate through action potentials along their axons, and those action potentials are electrical events that depend on the movements of ions, particularly sodium and potassium, across the neuronal cell membrane (Jefferys 2010). Postsynaptic conductance changes and the potential changes that accompany them alter the probability that an action potential will be produced in the postsynaptic cell. Postsynaptic potentials (PSPs) are called excitatory (or EPSPs) if they increase the likelihood of a postsynaptic action potential occurring, and inhibitory (or IPSPs) if they decrease this likelihood (Purves et al. 2001). Given that most neurons receive inputs from both excitatory and inhibitory synapses, it is important to understand more precisely the mechanisms that determine whether a particular synapse excites or inhibits its postsynaptic partner. In order to generate large EPSPs underlying depolarization shift (cause of interictal spike discharge or epileptic seizure), the normal small EPSPs must be amplified (Dichter and Ayala 1987). Blocking of chloride channel by non-competitive blockers at the picrotoxin convulsant site on $GABA_A$ receptors reduces IPSPs or increases the probability of firing of the neuron, causing an enhancement of excitatory postsynaptic action potentials (EPSPs) (Dichter and Ayala 1987).

Evidence Supporting this KER

Biological Plausibility

Seizure often involves the reorganizations occurring around the synapse that are extremely diverse and complex. What could be the functional consequences of synaptic reorganization? The hypotheses are admittedly very speculative, since we do not know the role of each parameter under physiological conditions. Very importantly, a drastic alteration of one parameter (e.g., the loss or reduction of GABAergic inhibition, or its transformation into excitation) may be without any functional impact (Bernard 2012). This is a key concept derived from the work of Prinz et al. (2004) performed in the stomatogastric system of the lobster, which has led to the concept that there are multiple solutions to a given biological problem. The stomatogastric system, which generates a rhythm vital for the animal, is composed of three nuclei connected to each other via different neurotransmitter systems. Knowing the types of channels expressed by the neurons in each nuclei and the type of connections, the researchers built a computer model in which each parameter (amplitude of the ionic current, strength of the connection) could take any biologically realistic value. They varied all the parameters, and selected the sets of parameters that produced the same rhythm recorded *in vivo*. They found that there are countless possible solutions, which produce the same behavior at the network level. Importantly, they also found that the system is "resistant" even if one type of channel is not expressed, or if a connection between two nuclei is missing. Further, the values taken by a given parameter (among the sets of solutions) match the biological variability (Schulz et al. 2007; Marder and Goaillard 2006). That is, the variability of a given parameter measured in a biological system (e.g. amplitude of $GABA_A$ receptor-mediated currents) may just reflect the different solutions that enable networks to function adequately. One might therefore consider that all the modifications occurring in epileptic networks may simply constitute the expression of another set of "solutions" to perform normal physiological function. Seizures are, after all, very infrequent events, which suggests that most of the

time the system can cope with various parameters permutations without engaging in abnormal activity.

Nevertheless, important functional changes in epilepsy appear to stem from some of the synaptic modifications identified so far, including interictal activity (Bernard 2012). A shift to a depolarizing action in a minority of cells may be sufficient to favor the occurrence of interictal spikes (Cohen et al. 2002). Interictal-like activity appears very early after the initial insult in experimental models, and precedes (by days) and even predicts the appearance of the chronic phase of epilepsy defined by recurrent seizures (El-Hassar et al. 2007; Williams et al. 2009; White et al. 2010). Using a crude model of hippocampal circuitry, El-Hassar et al. (2007) have tried to determine the conditions sufficient for the genesis of interictal spikes. Many different solutions exist, which include decreased dendritic GABAergic inhibition and increased glutamatergic excitation (El-Hassar et al. 2007), in a range of values found experimentally (El-Hassar et al. 2007). This model does not explain why interictal activity is not permanent in vivo, but suggests clues regarding its underlying mechanisms. Since interictal activity is rarely encountered in non-epileptic individuals, it has been proposed that it is pathological. Studies performed *in vitro* suggest that interictal-like activity can produce long-term potentiation of synapses, thus contributing to the construction of hyperexcitable networks (Dzhala and Staley 2003). The presence of interictal-like activity during the earliest stages of epileptogenesis may not only constitute biomarkers for at-risk patients, but also one core mechanism of epileptogenesis (El-Hassar et al. 2007; Williams et al. 2007; White et al. 2010). One study performed in patients with temporal lobe epilepsy suggests that the size of the epileptogenic zone increases with the duration of epilepsy (Bartolomei et al. 2008). The brain regions outside the epileptogenic zone (i.e. the irritative zone) are often characterized by the presence of interictal spikes. Some of these regions become part of the epileptogenic zone as epilepsy evolves in time (Bartolomei et al. 2008). It is therefore tempting to propose that interictal spikes participate in the transformation of the irritative regions into epileptic ones.

Empirical Evidence

There are numerous studies that reported the linkage between inhibitory postsynaptic potential (IPSP) reductions and enhanced excitatory postsynaptic potentials (EPSPs). For instance, Li et al. (1999) examined synaptic potentials of neurons in inferior colliculus (IC) cortex slice and the roles of GABA and glutamate receptors in generating these potentials. Using a $GABA_A$ antagonist, they blocked IPSPs and paired pulse inhibition of EPSPs (leading to amplified EPSPs) in the electrical stimulation of the IC commissure that elicited only IPSPs (10% of cells), only EPSPs (51%), or both (38%).

Uncertainties and Inconsistencies

A synapse involves three compartments: the presynaptic terminal, the postsynaptic site, and the glial cell processes surrounding them. Many features of the synapse can be modified. The number of synapses established by a given neuron on its targets can decrease ("pruning" or death of the presynaptic neuron) or increase (sprouting, neosynaptogenesis). The properties of the presynaptic terminal can be changed (release probability, neurotransmitter concentration in vesicles, control by presynaptic receptors). On the postsynaptic site, the number, subunit composition, and function (e.g., phosphorylation, anchoring) of the receptors can be changed. Finally, alterations at the glial cell level may affect the environment of the synapse and its function (neurotransmitter uptake, energy supply to neurons, etc.). See Bernard (2012) for a detailed review on the state of our current knowledge regarding the time-dependent reorganizations of GABAergic circuits at the

synaptic level during epileptogenesis and a discussion on the possible functional consequences of these alterations (particularly on the fate of GABAergic circuits).

With respect to the cause of EPSP amplification, there exist at least four mechanisms other than the withdrawal or reduction of inhibition in the epileptic foci of normal CNS (Dichter and Ayala 1987): (i) frequency potentiation of EPSPs, (ii) changes in the space constant of the dendrites (or spines) of the postsynaptic neuron, (iii) activation of the NMDA receptor as the cell depolarizes as a result of a reduction in voltage-dependent block of the receptor by Mg^{2+}, and (iv) potentiation by neuromodulators that are released during the ID (for example, norepinephrine, somatostatin, and acetylcholine). In addition to direct increases in excitatory synaptic efficacy, the depolarizing effects of EPSPs can be supplemented by several voltage-dependent intrinsic currents that exist in CNS neurons. These include slowly inactivating Na^{+} and Ca^{2+} currents and a large, transient Ca^{2+} current that is likely to be responsible for Ca^{2+}-dependent action potentials.

The above synaptic modifications and diverse causes of EPSP amplification constitute the uncertainty factors for the relationship between reduced IPSPs and amplification of EPSPs at the affected GABAergic neurons.

References

Bartolomei F, Chauvel P, Wendling F. 2008. Epileptogenicity of brain structures in human temporal lobe epilepsy: a quantified study from intracerebral EEG. Brain. 131:1818–30.

Bernard C. 2012. Alterations in synaptic function in epilepsy. In: Noebels JL, Avoli M, Rogawski MA, et al. (Eds), Jasper's Basic Mechanisms of the Epilepsies [Internet]. 4th edition. Bethesda (MD): National Center for Biotechnology Information (US); Website: http://www.ncbi.nlm.nih.gov/books/NBK98161/.

Cherubini E, Conti F. 2001. Generating diversity at GABAergic synapses. Trends Neurosci. 24:155–162

Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. 2002. On the origin of interictal activity in human temporal lobe epilepsy in vitro. Science. 298:1418–21.

Cossart R, et al. 2001. Dendritic but not somatic GABAergic inhibition is decreased in experimental epilepsy. Nat Neurosci. 4:52–62.

Dichter MA, Ayala GF. 1987. Cellular mechanisms of epilepsy: A status report. Science 237:157-64.

Dzhala VI, Staley KJ. 2003. Transition from interictal to ictal activity in limbic networks in vitro. J Neurosci. 23:7873–80.

El-Hassar L, et al. 2007. Cell domain-dependent changes in the glutamatergic and GABAergic drives during epileptogenesis in the rat CA1 region. J Physiol. 578:193–211.

Farrant M, Nusser Z. 2005. Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. Nat Rev Neurosci. 6:215–29.

Jefferys JGR. 2010. Advances in understanding basic mechanisms of epilepsy and seizures. Seizure 19:638–46.

Jentsch TJ, Stein V, Weinreich F, Zdebik AA. 2002 Molecular Structure and Physiological Function of Chloride Channels. Physiol Rev. 82(2):503-68.

Li Y, Evans MS, Faingold CL. 1999. Synaptic response patterns of neurons in the cortex of rat inferior colliculus. Hear Res. 137(1-2):15-28.

Marder E, Goaillard JM. 2006. Variability, compensation and homeostasis in neuron and network function. Nat Rev Neurosci. 7:563–74.

Prescott SA. 2014. Chloride channels. In: Jaeger D and Jung R (Eds.), Encyclopedia of Computational Neuroscience, Springer, New York, 2014. pp.1-4.

Prinz AA, Bucher D, Marder E. 2004. Similar network activity from disparate circuit parameters. Nat Neurosci. 7:1345–52.

Purves D, Augustine GJ, Fitzpatrick D, et al. (Eds). 2001. Chapter 7. Neurotransmitter Receptors and Their Effects. In: Neuroscience (2nd edition). Sunderland (MA): Sinauer Associates. Website: http://www.ncbi.nlm.nih.gov/books/NBK10799/

Schulz DJ, Goaillard JM, Marder EE. 2007. Quantitative expression profiling of identified neurons reveals cell-specific constraints on highly variable levels of gene expression. Proc Natl Acad Sci USA. 104:13187–91.

White A, et al. 2010. EEG spike activity precedes epilepsy after kainate-induced status epilepticus. Epilepsia. 51:371–83.

Williams PA, et al. 2009. Development of spontaneous recurrent seizures after kainate-induced status epilepticus. J Neurosci. 29:2103–12.

Relationship: 684: Generation, Amplified excitatory postsynaptic potential (EPSP) leads to Occurrence, A paroxysmal depolarizing shift

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Binding to the picrotoxin site of ionotropic GABA receptors leading to epileptic seizures in adult brain	adjacent	Moderate	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI
rat	Rattus norvegicus	High	NCBI

Numerous studies have documented experimental evidence in support of this relationship even though the underlying mechanisms are still not completely understood. See reviews of Bromfield et al. (2006) and Dichter and Ayala (1987) for studies using rat or human tissues or cell lines as the experimental subject.

Key Event Relationship Description

Blockage of the ion channel of the iGABAR causes membrane depolarization and a reduction in inhibitory postsynaptic currents. This leads to the increased, abnormal neuron firing that causes a wave of depolarization throughout the brain/neuronal tissue. At the level of single neurons, epileptiform activity consists of sustained neuronal depolarization resulting in a burst of action potentials, a plateau-like depolarization associated with completion of the action potential burst, and then a rapid repolarization followed by hyperpolarization. This sequence is called the paroxysmal depolarizing shift. The bursting activity resulting from the relatively prolonged depolarization of the neuronal membrane is due to influx of extracellular Ca^{2+}, which leads to the opening of voltage-dependent Na^{+} channels, influx of Na^{+}, and generation of repetitive action potentials. The subsequent hyperpolarizing afterpotential is mediated by iGABA receptors and Cl^{-} influx, or by K^{+} efflux, depending on the cell type (Bromfield et al 2006).

Evidence Supporting this KER

Biological Plausibility

It has been proposed that as the potentiated EPSP begins to depolarize the neuron, a threshold is reached for the development of a slowly inactivating Na^+ current that amplifies the depolarization. As depolarization continues, the low threshold Ca^{2+} current may turn on to further depolarize the neuron, while NMDA-mediated excitatory synapses become more effective. Eventually, both higher threshold Na^+ and Ca^{2+} currents are activated, and the neuron discharges with a burst of action potentials and an additional slow depolarization (Herron et al. 1985; Dingledine et al. 1986). This hypothesis involves the interplay of both synaptic and voltage-dependent intrinsic events that occur in normal central neurons.

An alternative hypothesis for PDS generation focuses more on changes in the intrinsic properties of neurons resulting in the development of burst firing independent of a primary change in synaptic interactions (Dichter and Ayala 1987).

Empirical Evidence

For the first hypothesis, Higashida and Brown (1986) and Madison et al. (1986) have demonstrated that epilepsy occurs when the usual balance of these normal events is altered by a change in synaptic efficacy or a change in the control of intrinsic membrane currents. The reason that any given form of epilepsy may develop in a given brain region may depend on (i) differences in densities and locations of channels on various neurons, (ii) the interaction of intrinsic currents with one another and with synaptic currents under physiological conditions, (iii) the local synaptic organization of a given area, and (iv) the liberation of endogenous synaptic modulators that may alter the various voltage-dependent membrane currents through second messenger pathways.

For the alternative hypothesis, many studies have shown that in epilepsy models, this is most readily accomplished by inhibiting K^+ currents and by allowing the slower Ca^{2+} currents to be expressed (see review by Dichter and Ayala (1987)). Under more "natural" circumstances, a variety of possible mechanisms may contribute to the development of endogenous burst propensity in the absence of exogenous epileptogenic agents: (i) neuromodulators (acetylcholine, norepinephrine, and peptides) can reduce K^+ currents (although stimulation of endogenous pathways, even intensely, has not been shown to produce sufficient inhibition of K^+ currents to result in burst firing); (ii) both elevation of extracellular K ($[K^+]$) and reduction of extracellular Ca ($[Ca^{2+}]$) can change membrane characteristics and induce burst firing modes; and (iii) anatomical distortion and redistribution of channels after injury and partial denervation as seen in chronic epileptic foci may induce burst firing.

Uncertainties and Inconsistencies

In addition to the above two hypotheses with empirical evidence, some investigators have proposed that neurons with endogenous bursting characteristics must act as a pacemaker in order for epileptiform activity to develop (see review by Dichter and Ayala (1987)). Such neurons would be the CA2 and CA3 pyramidal cells in the hippocampus, layer IV and superficial layer V neocortical pyramidal cells, or the abnormally burst-firing neurons in chronic neocortical foci. This hypothesis is supported by the demonstration of the lower threshold for the induction of interictal discharges by epileptogenic agents in CA2 and CA3 and layer IV, the spread of abnormal activity from these areas to nearby areas in some

experimental foci, and by the correlation of the number of bursting cells with the seizure frequency in chronic foci.

However, this hypothesis has been challenged on theoretical grounds by models that demonstrate that a system with either positive or negative feedback elements does not require unstable individual elements in order to develop oscillating behavior. There is also experimental evidence against the obligatory involvement of neurons with endogenous burst-firing characteristics. Studies of *in vivo* hippocampal penicillin epilepsy and *in vitro* low Ca^{2+}-high K^{+} models of epilepsy indicate that area CAl is able to develop spontaneous IDs and seizures independent of areas CA2 and CA3. In addition, neocortical and spinal cord cultures, in which individual neurons do not discharge with intrinsic bursts, become organized into small synaptic networks that show synchronized "burst" behavior-all as a result of synaptic interactions. Thus it appears that endogenous, Ca^{2+}-dependent bursts are not strictly necessary for the development of synchronous bursting activity in a neural network, although their presence may be facilitatory and CNS regions containing such burst-firing neurons may have a particularly high epileptiform potential.

References

Bromfield EB, Cavazos JE, Sirven JI. 2006. Chapter 1, Basic Mechanisms Underlying Seizures and Epilepsy. In: An Introduction to Epilepsy [Internet]. West Hartford (CT): American Epilepsy Society; Available from: http://www.ncbi.nlm.nih.gov/books/NBK2510/.

Dichter MA, Ayala GF. 1987. Cellular mechanisms of epilepsy: A status report. Science 237: 157-164.

Dingledine R, Hynes MA, King GL. 1986. Involvement of N-methyl-D-aspartate receptors in epileptiform bursting in the rat hippocampal slice. J Physiol. 380:175-89.

Herron CE, Williamson R, Collingridge GL. 1985. A selective N-methyl-D-aspartate antagonist depresses epileptiform activity in rat hippocampal slices. Neurosci Lett. 61(3):255-60.

Higashida H, Brown DA. 1986. Two polyphosphatidylinositide metabolites control two K+ currents in a neuronal cell. Nature. 323(6086):333-5.

Madison DV, Malenka RC, Nicoll RA. 1986. Phorbol esters block a voltage-sensitive chloride current in hippocampal pyramidal cells. Nature. 321(6071):695-7.

Relationship: 630: Occurrence, A paroxysmal depolarizing shift leads to Occurrence, Epileptic seizure

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Binding to the picrotoxin site of ionotropic GABA receptors leading to epileptic seizures in adult brain	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

Most lines of supporting evidence come from studies using human and rodent epilepsy models. See Dichter and Ayala (1987) and Jefferys (2010) for examples.

Key Event Relationship Description

Dichter and Ayala (1987) reviewed our current understanding of the simple focal seizure models, where interictal discharge (ID) and seizures seem most closely related. In acute focal epilepsy, during the ID, thousands of neurons in the focus synchronously undergo an unusually large depolarization (the paroxysmal depolarizing shift or PDS), superimposed on which is a burst of action potentials. The PDS is followed by a hyperpolarizing potential (the post-PDS HP) and neuronal inhibition. In areas surrounding the focus, many neurons are inhibited during the ID. In distant projection areas, neurons can be excited briefly but more often are inhibited during the ID, according to their synaptic interactions. Axons that end within the focus generate action potentials, which can "backfire" and propagate antidromically. In addition, during the ID, at the site of the focus, extracellular levels of K^+ increase and levels of Ca^{2+} decrease, presumably because of exit of K^+ from and entry of Ca^{2+} into neuronal processes during the intense neuronal activity.

Evidence Supporting this KER

Biological Plausibility

As reviewed by Dichter and Ayala (1987), when seizures develop, at least in the acute focus, the neurons show a characteristic sequence of events: the post-PDS HP becomes smaller, gradually disappears, and is replaced by a depolarization, on top of which are smaller depolarizing waves that resemble small PDSs. This series of events occurs synchronously in the population of neurons within the focus, and the EEG develops after discharges (ADs) after several successive IDs. The ADs become longer with each ID and then progress into a seizure. Meanwhile, near and distant areas of brain are brought into the seizure process, and the abnormal activity spreads. During this process, levels of extracellular K^+ continue to increase until they reach a steady-state level well above normal, and levels of extracellular Ca^{2+} continue to decrease. Finally the seizure subsides, and the neuronal membrane hyperpolarizes well beyond control level. It is not known whether this orderly progression from IDs to seizures occurs in the same way in chronic epileptic foci or in many forms of human epilepsy.

Empirical Evidence

As summarized by Dichter and Ayala (1987), the EEG hallmarks of focal epilepsy both in animal models and in human epilepsy are the ictal, or seizure, discharge and the interictal spike discharge (ID). The EEG spike most often represents an electrophysiological marker for a hyperexcitable area of cortex and arises in or near an area with a high epileptogenic potential. As such, it has been considered the earliest and simplest electrical manifestation of the epileptic process and has been the target of extensive investigations. In some forms of epilepsy, seizure discharges can be seen to originate electrically and anatomically from the site of spike discharges, and the transition between spikes and seizures has been analyzed in these simple models. In other forms of epilepsy, however, the exact relation between spike discharge and the onset and localization of seizure discharges has been more difficult to determine. Depth electroencephalography from humans with focal epilepsy has demonstrated multiple patterns during the transition to seizure, only some of which resemble that seen in acute experimental focal epilepsy. Whether these observations indicate that the mechanisms underlying the transition in chronic human foci are different from those of the simple acute model is not yet clear.

Uncertainties and Inconsistencies

A crucial issue related to the development of the ID is how so many neurons within a focus develop simultaneous depolarizations. Synchronization may occur by any of several synaptic and nonsynaptic mechanisms: (i) recurrent synaptic excitation, (ii) antidromic activation of the afferent fibers, (iii) ephaptic interactions due to large currents that flow through extracellular spaces, (iv) changes in extracellular ionic concentrations, (v) electrical coupling between cortical neurons, and (vi) the diffuse liberation of modulators (Dichter and Ayala 1987). Two alternative hypotheses emerged, that could broadly be categorized as the epileptic neuron versus the epileptic network. In practice it really is impossible to divorce the two: epilepsy is essentially a collective phenomenon that requires synchrony amongst large numbers of neurons, but the reason for the excessive synchrony and excitation can be abnormal intrinsic properties (the epileptic neuron), or abnormal circuitry (the epileptic network), or both (in most cases) (Jefferys 2010).

References

Dichter MA, Ayala GF. 1987. Cellular mechanisms of epilepsy: A status report. Science 237: 157-164.

Jefferys JGR. 2010. Advances in understanding basic mechanisms of epilepsy and seizures. Seizure 19:638–46.