AOP ID and Title:

SNAPSHOT

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AOP 10: Binding to the picrotoxin site of ionotropic GABA receptors leading to epileptic seizures

Short Title: Blocking iGABA receptor ion channel leading to seizures

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Status

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Abstract

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 of vertebrates, iGABARs have also been described in many different phyla of invertebrates such as social amoeba (*Dictyostelium discodeum*), cnidarians, mollusks, annelids, arthropods, nematodes, and chordates. Chemical interactions with iGABARs can cause a variety of pharmacological and neurotoxicological effects depending on the location of the active or allosteric site affected. Interactions at three different types of binding sites on iGABARs can antagonize the postsynaptic inhibitory functions of GABA and lead to epileptic seizures and death. One of the three types of binding sites is the picrotoxin convulsant site located inside of the iGABAR pore that spans neuronal cell membranes. This AOP begins with a molecular initiating event (MIE) where chloride conductance through the ion channel is blocked due to chemical binding at or near the central pore of the receptor complex (e.g., the picrotoxin site). As a result, the first key event is a decrease in inward chloride conductance through the ligand-gated ion channel. This leads to the second key event, 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). Although the underlying mechanism for the large EPSP is not well

understood, a spike (rise) of intracellular Ca2+ is 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-depolarlization hyperpolarization becomes smaller, gradually disappears, and is replaced by a depolarization. This characteristic depolarization-shrinking hyperpolarization sequent of events represents the fourth key event 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 is 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 brain.

Summary of the AOP

Stressors

Name	Evidence
Picrotoxin	Strong
Lindane	Strong
Dieldrin	Strong

Picrotoxin

Picrotoxin seizures are well defined mechanistically. They arise from $GABA_A$ receptor chloride channel blockade. (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 prposed model for channel binding sites. Neurochem Int. 50(1): 61-8.)

Lindane

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

Dieldrin

See evidence text for picrotoxin and lindane.

Molecular Initiating Event

Title	Short name
Binding at picrotoxin site, iGABAR chloride channel	Binding at picrotoxin site, iGABAR chloride channel

667: Binding at picrotoxin site, iGABAR chloride channel

Short Name: Binding at picrotoxin site, iGABAR chloride channel

AOPs Including This Key Event

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

Stressors

Name
Picrotoxin
Lindane
Fipronil
RDX
Alpha-endosulfan
Penicillin

Evidence for Perturbation of this Molecular Initiating Event by Stressor

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 binding site in the chloride channel lumen lined by five TM2 segments. 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).

Biological Organization

Level of Biological Organization
Molecular

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Strong	NCBI
rat	Rattus norvegicus	Strong	NCBI
fruit fly	Drosophila melanogaster	Strong	NCBI
mouse	Mus musculus	Strong	NCBI
dogs	Canis lupus familiaris	Strong	NCBI

Life Stage Applicability

Life Stage	Evidence
Adult	Strong

Sex Applicability

Sex	Evidence
Unspecific	Strong

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).

How this Key Event Works

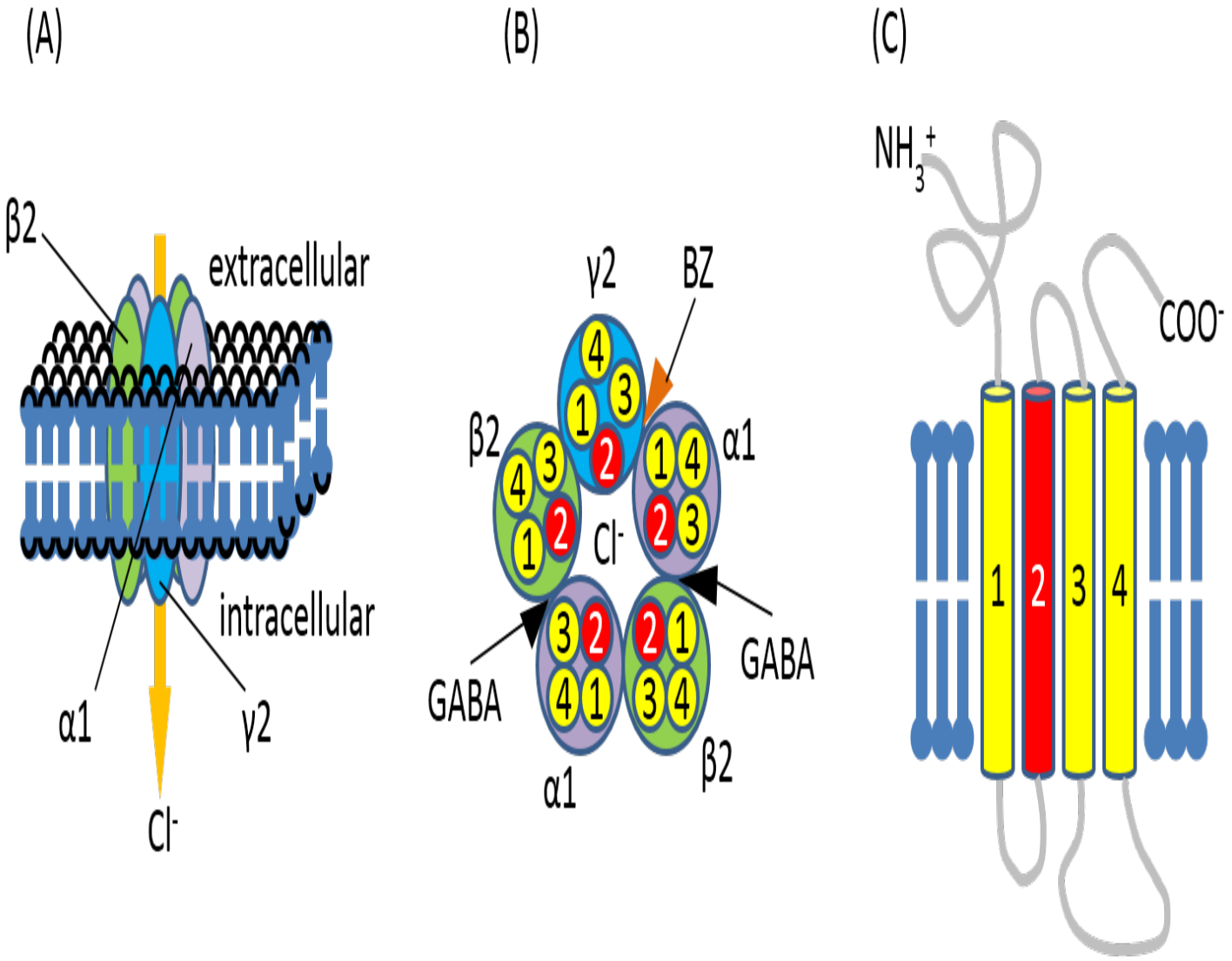

Figure 1. Structure of ionotropic GABA receptors based on the consensus in multiple literature reviews (Source: Gong et al. 2015). Shown is a common subtype α1β2γ2 of GABAA receptors found in the mammalian CNS. (A) Five subunits from three subunit subfamilies assemble to form a heteropentameric chloride 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. 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, non-competitive channel blockers (e.g., fipronil, lindane, picrotoxin 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).

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).

References

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Key Events

Title	Short name
Reduction, Ionotropic GABA receptor chloride channel conductance	Reduction, Ionotropic GABA receptor chloride channel conductance
Occurrence, A paroxysmal depolarizing shift	Occurrence, A paroxysmal depolarizing shift
Reduction, Neuronal synaptic inhibition	Reduction, Neuronal synaptic inhibition
Generation, Amplified excitatory postsynaptic potential (EPSP)	Generation, Amplified excitatory postsynaptic potential (EPSP)

64: Reduction, Ionotropic GABA receptor chloride channel conductance

Short Name: Reduction, Ionotropic GABA receptor chloride channel conductance

AOPs Including This Key Event

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

Biological Organization

Level of Biological Organization
Cellular

Evidence Supporting Applicability of this Event

Taxonomic Applicability

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

Banerjee et al. (1999) reported functional modulation of GABA-A 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 GABA(A) receptor function by 3 µM dieldrin using the GABA-induced (36)Cl(-) uptake method.

Bromfield et al. (2006) reviewed evidence for GABA-A receptors in human and mammalian brains.

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

How this Key Event Works

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). A non-competitive channel blocker binds at or near the central pore of the receptor complex (i.e., the picrotoxin site) and directly blocks chloride flux through the ion channel (Gong et al. 2015)

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.

References

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616: Occurrence, A paroxysmal depolarizing shift

Short Name: Occurrence, A paroxysmal depolarizing shift

AOPs Including This Key Event

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

Biological Organization

Level of Biological Organization
Tissue

Evidence Supporting Applicability of this Event

Taxonomic Applicability

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

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

How this Key Event Works

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 non synchronous 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".

Here, 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 (PDS). The bursting activity resulting from the relatively prolonged depolarization of the neuronal membrane is due to influx of extracellular Ca2+, 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 methods or *in vivo* using electroencephalography techniques (Niedermeyer and da Silva 2005).

References

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669: Reduction, Neuronal synaptic inhibition

Short Name: Reduction, Neuronal synaptic inhibition

AOPs Including This Key Event

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

Biological Organization

Level of Biological Organization
Cellular

Evidence Supporting Applicability of this Event

Taxonomic Applicability

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

human	Homo sapiens	Strong	NCBI
Japanese quail	Coturnix japonica	Strong	NCBI

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 GABA-A 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).

How this Key Event Works

A decline in conductance through chloride channels in iGABARs causes a reduction in GABA-mediated inhibition of neuronal synaptic signalling, which 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

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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.

682: Generation, Amplified excitatory postsynaptic potential (EPSP)

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

AOPs Including This Key Event

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

Biological Organization

Level of Biological Organization
Tissue

Evidence Supporting Applicability of this Event

Taxonomic Applicability

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

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.

How this Key Event Works

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 using intracellular electrodes. See Miura et al. (1997) and Bromfield et al. (2006) for details.

References

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Adverse Outcomes

Title	Short name
Occurrence, Epileptic seizure	Occurrence, Epileptic seizure

613: Occurrence, Epileptic seizure

Short Name: Occurrence, Epileptic seizure

AOPs Including This Key Event

AOP ID and Name	Event Type
10: Binding to the picrotoxin site of ionotropic GABA receptors leading to epileptic seizures	AdverseOutcome

Biological Organization

Level of Biological Organization
Individual

Evidence Supporting Applicability of this Event

Taxonomic Applicability

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

mouse	Mus musculus	Strong	NCBI
honeybee	Apis mellifera	Strong	NCBI
eisenia fetida	eisenia fetida	Strong	NCBI

A wide range of species including invertebrates and vertebrates have been documented (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).

How this Key Event Works

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 hyperexicitation 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).

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. Repetitive discharges lead to: 1) an increase in extracellular K+, which blunts the extent of hyperpolarizing outward K+ currents, tending to depolarize neighboring neurons; 2) accumulation of Ca++ in presynaptic terminals, leading to enhanced neurotransmitter release; and 3) depolarization-induced activation of the NMDA subtype of the excitatory amino acid receptor, which causes more Ca++ influx and neuronal activation. 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.

Regulatory Examples Using This Adverse Outcome

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.

References

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Scientific evidence supporting the linkages in the AOP

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

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

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

How Does This Key Event Relationship Work

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.

Weight of Evidence

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 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 Support for Linkage

Numerous pharmacological and computational studies have lent strong support of 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 or 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.

Quantitative Understanding of the Linkage

Is it known how much change in the first event is needed to impact the second? There is no study that quantitatively measured both receptor binding affinity and inhibition of chloride flux.

Are there known modulators of the response-response relationships? There is no known modulator that acts in between receptor binding and channel blocking, even though there are many binding sites other than the picrotoxin-binding sites that may affect chloride conductance.

Are there models or extrapolation approaches that help describe those relationships? No, however, there exist computational models based on 3D structure modeling that have been used to predict the binding affinity of ligands/chemicals at specific pockets of the ion channel (Yoon et al. 1993; Zheng et al. 2014).

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

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	Strong	NCBI
guinea pig	Cavia porcellus	Strong	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).

How Does This Key Event Relationship Work

A decline in conductance through chloride channels in iGABARs causes a reduction in GABA-mediated inhibition of neuronal synaptic signalling, 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

Weight of Evidence

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 Support for Linkage

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 or 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.

Quantitative Understanding of the Linkage

Is it known how much change in the first event is needed to impact the second? Yes, but very few studies reported changes in both events. One examples is Williams et al. (2011), where whole-cell in vitro recordings in the rat basolateral amygdala (BLA) showed that RDX reduced the frequency and amplitude of spontaneous GABA(A) receptor–mediated inhibitory postsynaptic currents and the amplitude of GABA-evoked postsynaptic currents, whereas in extracellular field recordings from the BLA, RDX induced prolonged, seizure-like neuronal discharge.

Are there known modulators of the response-response relationships? There is no known modulator that acts in between chloride channel conductance decrease and neuronal inhibition reduction, even though there are many other players such as potassium-chloride cotransporters and sodium-potassium-chloride cotransporters that may affect chloride flux/homeostasis and electrochemical gradient (Prescott 2014), leading to changes in postsynpatic neuronal inhibition.

Are there models or extrapolation approaches that help describe those relationships? No.

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Occurrence, A paroxysmal depolarizing shift leads to Occurrence, Epileptic seizure

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links

human	Homo sapiens	Strong	NCBI
rat	Rattus norvegicus	Strong	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.

How Does This Key Event Relationship Work

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 anntiromically. In addition, during the ID, at the site of the focus, extracellular levels of K+ increase and levels of Ca2+ decrease, presumably because of exit of K+ from and entry of Ca2+ into neuronal processes during the intense neuronal activity.

Weight of Evidence

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 Ca2+ 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 Support for Linkage

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 or Inconsistencies

A cricial 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 (in most cases?) both (Jefferys 2010).

Quantitative Understanding of the Linkage

Is it known how much change in the first event is needed to impact the second? No quantitative relationship has yet been established between these two key events.

Are there known modulators of the response-response relationships? Yes. For detailed description of known modulators, see Dichter and Ayala (1987).

Are there models or extrapolation approaches that help describe those relationships? Yes. For more information on different models and hypotheses, see Dichter and Ayala (1987) and Jeffreys (2010).

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

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

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

How Does This Key Event Relationship Work

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 neuronl 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 communicated through action potentials along their axons, and those action potentials were electrical events that depended 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).

Weight of Evidence

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 lead 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 Support for Linkage

There are numerous studies that reported the linkage between inhibitory postsynaptic potential (IPSP) reduction 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 or 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 withdrawl or reduction of inhibtion 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 ofthe NMDA receptor as the cell depolarizes as a result of a reduction in voltage-dependent block of the receptor by Mg2+, 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 Ca2+ currents and a large, transient Ca2+ current that is likely to be responsible for Ca2+-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.

Quantitative Understanding of the Linkage

Is it known how much change in the first event is needed to impact the second? Inhibitory presynaptic neurons release neurotransmitters that then bind to the postsynaptic receptors; this induces a postsynaptic conductance change as ion channels open or close. An electric current that changes the postsynaptic membrane potential to create a more negative postsynaptic potential is generated. Depolarization can also occur due to an IPSP if the reverse potential is between the resting threshold and the action potential threshold.

Are there known modulators of the response-response relationships? Yes. See Bernard (2012) for discussion on synaptic modifications and consequent functional alterations in epilepsy.

Are there models or extrapolation approaches that help describe those relationships? Yes. See Prinz et al. (2004) and El-Hassar et al. (2007) for details and above for brief descriptions.

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

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Strong	NCBI
rat	Rattus norvegicus	Strong	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.

How Does This Key Event Relationship Work

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 Ca2+, 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).

Weight of Evidence

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 Ca2+ current may turn on to further depolarize the neuron, while NMDA-mediated excitatory synapses

become more effective. Eventually, both higher threshold Na+ and Ca2+ 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 Support for Linkage

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 voltagedependent 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 Ca2+ 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 ([Ca2+]) 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 or 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-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 ofsynaptic interactions. Thus it appears that endogenous, Ca2+-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.

Quantitative Understanding of the Linkage

Is it known how much change in the first event is needed to impact the second? No quantitative relationship has

been established between the two key events.

Are there known modulators of the response-response relationships? Yes. There are many modulators documented (see Dichter and Ayala (1987) for review).

Are there models or extrapolation approaches that help describe those relationships? Several different models have been proposed (see Dichter and Ayala (1987) for review).

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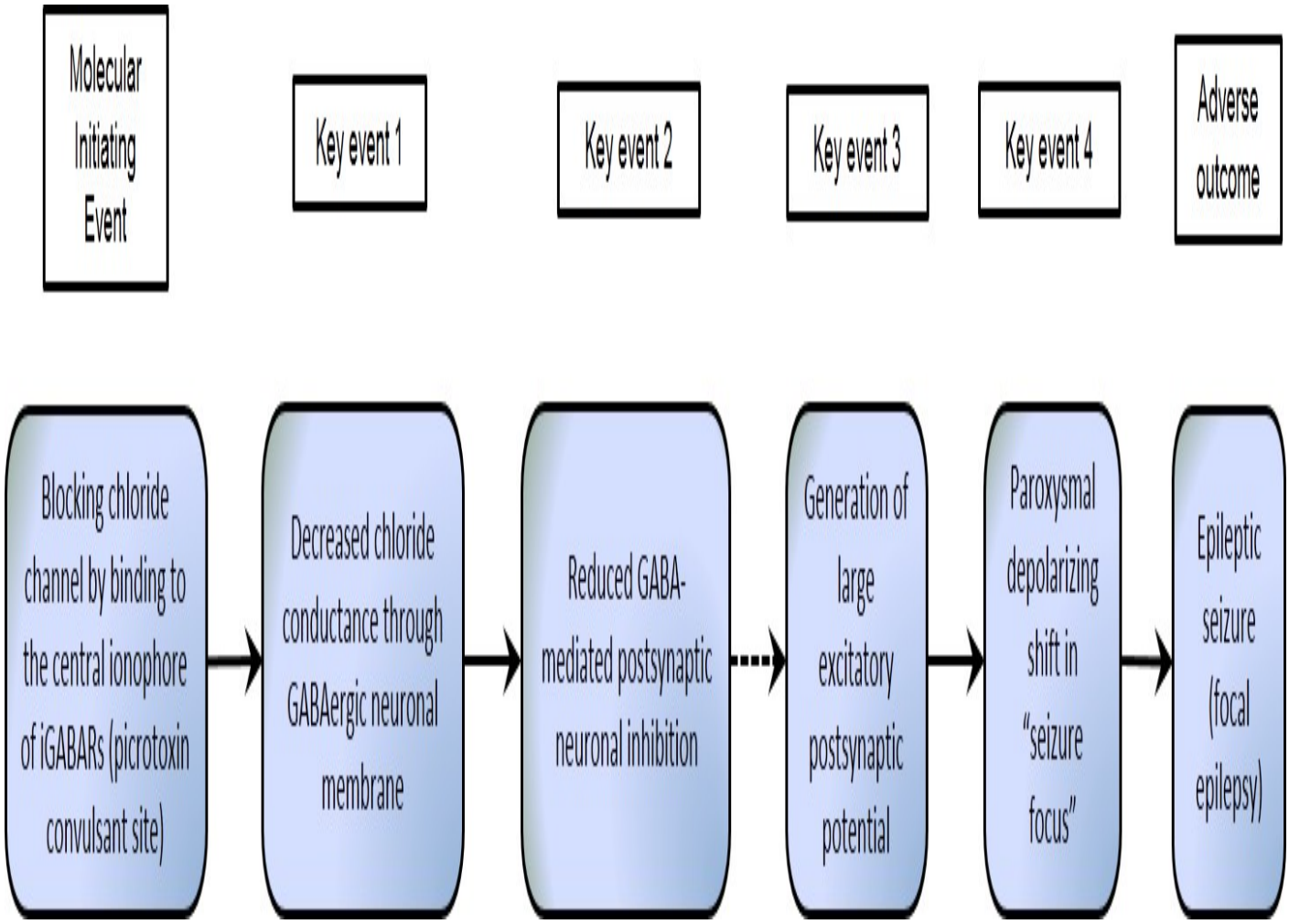
Graphical Representation



Overall Assessment of the AOP

Biological plausibility

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). 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). 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 GABA antagonists including 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 electrophysiresponse 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). 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 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 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. Repetitive discharges lead to: 1) an increase in extracellular K+, which blunts the extent of hyperpolarizing outward K+ currents, tending to depolarize neighboring neurons; 2) accumulation of Ca++ in presynaptic terminals, leading to enhanced neurotransmitter release; and 3) depolarization-induced activation of the NMDA subtype of the excitatory amino acid receptor, which causes more Ca++ influx and neuronal activation. 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).

Uncertainties, inconsistencies, and data gaps

No inconsistencies have been reported so far, though some uncertainties and data gaps do exist. For instance, it is less well understood about 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). 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 not yet fully understood (Dichter and Ayala 1987).

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Adults	Strong

Taxonomic Applicability

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

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

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 because GABA acts as an excitatory neurotransmitter due to increased intracellular Cl^- concentration during development of the nervous system (Taketo and Yoshioka 2000). 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

See Collier et al. (2016) for details.

Data Quality Criteria	Weight	MIE		KE 1		KE 2		KE 3		KE 4		AO	
		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**

Causal Linkage Criteria	Weight	MIE to KE 1		KE 1 to KE 2		KE 2 to KE 3		KE 3 to KE 4		KE 4 to AO	
		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 GABAA receptor function to 55% of control, as measured by the GABA-induced 36Cl- 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 possessing 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).

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