| **Section** | **Reviewer comment** | **Author response (précis)** |
| --- | --- | --- |
| **Name** | OK ("Binding to the picrotoxin site of ionotropic GABA receptors leading to epileptic seizures") |  |
| **Authors** | OK (Ping Gong, Ed Perkins) |  |
| **Date** | OK (snapshot 2017-02-09 13:22) |  |
| **Abstract** | SR1: The abstract does clear describe the content of the AOP. However, each KE is described at a higher level of detail then generally required for an abstract. Therefore I would suggest that it could be written more concisely (is there a word limit for abstracts? This one is nearly 500 words).  SR2: It describes the content but isn’t concise because it starts with a lot of background information on GABA-A receptors and binding sites that arguably aren’t strictly necessary to describe the specific AOP being developed. | We have removed the background information and revised the abstract which is now reduced to 343 words. The word limit of abstract is 200-400 words according to the online instructions for Abstract provided at the AOPwiki website. We have created a new but optional “Background” section where we introduced iGABAR and its classification. |
| **MIE** | **KE667: Binding at picrotoxin site, iGABAR chloride channel"** |  |
|  | PR: Might be better renamed to "Postsynaptic GABAAR antagonism"; the term ionotropic GABA (iGABA) receptor could cause confusion. The only PubMed hit for iGABAR is a 2015 paper by the present AOP authors… The standard receptor nomenclature is GABA-A receptor (>23000 PubMed hits). The identity of the "picrotoxin site" is not clear.  SR1: Use of iGABAR instead of GABA-a is unusual. | The term iGABAR is not new, nor did we coin it. Although it is not as popular as GABAAR, a key word search in PubMed results in 1235 hits. We prefer the use of iGABAR over GABAAR because the former covers both vertebrates and invertebrates. We added some information about the classification of GABA receptors in the newly created Background section. |
|  | PR: The identity of the "picrotoxin site" is not clear. I could not find any clear definition or molecular characterization of a "picrotoxin site" in the literature; the cited paper by Carpenter et al 2013 refers to 2 sites (major interface pocket and minor allosteric conformational change site).  SR1: Minor concerns that the “picrotoxin site” might be too chemical-specific. Can this be generalized? | Although Carpenter et al (2013) reported evidence for a secondary picrotoxin-binding site, the primary location of this site has been well-documented and described.  The picrotoxin-binding site is a pharmacologically well-defined term, and it is not associated with any specific chemical. See Figure 5 in Hibbs RE, Gouaux E. Principles of activation and permeation in an anion-selective Cys-loop receptor. Nature. 2011 Jun 2;474(7349):54-60). |
|  | PR: After browsing the literature, I see that it is still unclear at a molecular level how individual chemicals block GABAAR Cl- influx (for example, Naffaa MM, Samad A. The binding mode of picrotoxinin in GABAA-ρ receptors: Insight into the subunit's selectivity in the transmembrane domain. Comput Biol Chem. 2016 Oct;64:202-209). An additional problem is that you are naming a specific chemical in the MIE | Naffaa and Samad (2016) describes the subunit selectivity for the binding mode of picrotoxinin as non-competitive antagonist in GABAA-p receptors, implying that picrotoxin not only can bind competitively to the picrotoxin-binding site, but also display a non-competitive binding mode. This paper only dealt with one chemical picrotoxinin, and it did not suggest any general ambiguity for the MIE of non-competitive binding to picrotoxin site. As a matter of fact, there are so many different subtypes of iGABAR (especially GABAAR), the picrotoxin-binding site is not equally well-defined in every subtype. The GABAA-p subtype is one of the less well-defined subtypes. The reviewer may be confused between picrotoxin-binding site and the possible binding sites and modes of the chemical picrotoxinin. |
|  | SR2: As written, the MIE is defined by binding to a particular pharmacologically defined binding site (the picrotoxin site) and the known ability to ability inhibit the Cl conductance. [….] Under the current definition, and strictly speaking the MIE is applicable to organisms if and only if those organisms have GABA-AR and that GABA-AR has a picrotoxin binding site. So some additional definition of the applicability in this context would be helpful. | We agree with this reviewer. In fact, we stated in the section “Evidence Supporting Taxonomic Applicability” that “Theoretically, this MIE is applicable to any organisms that possess ionotropic GABA receptors (iGABARs) in their central and/or peripheral nervous systems”. We also provided evidence to support the ubiquitous existence of iGABARs in both vertebrates and invertebrates. |
|  | PR: An alternative possibility would be to define the MIE in terms of Cl- channel inhibition (so use KE64 as the MIE). It doesn't matter exactly where in the ion channel a chemical is binding, as long as it blocks it. Using a more generic functional MIE could broaden the applicability of the AOP to other proconvulsant GABAAR antagonists such as bicuculline, securinine, metrazol, flumazenil. | As summarized in our recent review (Gong et al., 2015), there are at least three types of ligand binding that may cause postsynaptic iGABAR (GABAAR included) antagonism. So, we believe that it is the best not to merge all three MIEs due to their distinct mode of action, despite that they converge on the downstream key events.  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 (this MIE). 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). |
|  | SR2: This represents the classic chunking/splitting problem in describing key events because a more generic concept such as “binding to the GABA-AR at an allosteric site” would also be acceptable but would then require the different pharmacologically defined allosteric sites be included in another way, for example via difference ways to measure the binding interaction. I also wonder whether we need an explicit molecular level key event of Cl channel blocking because it is conceivable that chemicals could be made that inhibit binding of some ligands at that site and yet do not themselves inhibit the conductance channel. | As we explained above, there are three well-defined and distinct types of antagonistic ligand-receptor interactions that characterize three MIEs/AOPs. Since iGABAR include GABAAR, we foresee that only three iGABAR antagonism-based MIEs/AOPs will be generated (including the present MIE/AOP). From the structural biology point of view, there exist binding sites in a receptor (protein) that display either inhibitory or excitatory effects. A chemical/drug can be introduced to compete for these sites with GABA or other indigenous neurotransmitters, exerting downstream impact via inhibiting or increasing conductance. |
|  | PR: The measurement/detection text appears to be more on binding characteristics (docking/pharmacophore) than functional inhibition [….] More targeted guidance might be helpful, e.g. Atack JR et al, Br J Pharmacol. 2007;150:1066. | We provided a reference (Chen et al. 2006) that covers nearly all methods. We believe that we should avoid lengthy descriptions of technical methods for an AOP. The reference mentioned by the PR (Atack et al. 2007) is one of the specific method applied to a specific study. We have added this reference as an example to explain that one should search for specific methods for specific application. |
|  | SR2: I don’t see any guidance on how comprehensive the measurement/detection section needs to be. Perhaps the point is to encourage crowd sourcing to fill in other ways of measuring this? | Our take is that an AOP should be considered as a review rather than a research paper. Hence, we did not provide detailed technical descriptions of methods for measuring or analyzing the MIE. |
|  | PR: Some links need correcting, e.g snapshot p.3, 667: "http://aopwiki.ag.epa.gov/events/667" should be "http://aopwiki.org/events/667" | We did not make the snapshot. If an updated new snapshot is required, we may provide one after this round of review. |
| **KEs** | **KE64: Reduction, Ionotropic GABA receptor chloride channel conductance** |  |
|  | PR: Maybe rename this KE to "Postsynaptic GABAAR antagonism" and use as MIE? It doesn't matter exactly where in the ion channel a chemical is binding, as long as it blocks it. Using a more generic functional MIE could broaden the applicability of the AOP to other proconvulsant GABAAR antagonists. | There are at least three types of ligand binding that may cause postsynaptic iGABAR (GABAAR included) antagonism. Each of these three types of binding corresponds to an individual MIE. We believe that it is the best not to merge all three MIEs due to their distinct mode of action, despite that they converge on the downstream key events. |
|  | PR: Biological context text is OK, but I get no information from the clickable NCBI links. More informative would be for example [uniprot.org link]. | The web link provided by the PR leads to the protein sequences of GABAA receptors of many organisms curated in Uniprot. This is another evidence of the ubiquitous distribution of iGABAR, which, however, is irrelevant to KE64. |
|  | SR1: Description of this KE has too much overlap with MIE. Please describe this KE in terms of “conductance” only… and not what is “causing” the reduced conductance (i.e. the MIE). | We agree, and have deleted the sentence “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)”. |
|  | SR2: As described this is the cellular aggregate response due to inhibition of some quantity of the individual molecular chloride channels. So also including a placeholder KE with the same effect at the molecular level might encourage crowd sourcing to fill in the detail of the key event relationship between how much binding at the molecular level leads to the cellular response? | We disagree with SR2 on a placeholder intermediate KE because KE64 is already well understood and a dose-dependent relationship is well documented. |
|  | PR: Cited measurement methods are arguably over-specific to neural tissue (and low-throughput). Suggest a higher level citation to aid the user.  SR1: The measurement methods provided are appropriate and briefly described and cited. The biological context is also appropriately discussed and cited.  SR2: Methods could be expanded, e.g., what about the high throughput channel measurements in FLIPR, FDSS or Ion Works technologies? | Author High throughput screening (HTS) assays are designed for a wide range of ion channels at the screening level; they are not specific enough and are not suitable for mechanistic studies. |
|  | **KE669: Reduction, Neuronal synaptic inhibition.** |  |
|  | PR: Maybe a more precise title would be "Reduction, Neuronal presynaptic inhibition"? Evidence cited for a single chemical - RDX (Williams et al 2011). Is it really necessary to have this as a separate KE from KE682? Maybe combine? | This is a well-defined event before KE682 with well-defined endpoints, i.e., reduced frequency and amplitude of GABAA receptor mediated spontaneous inhibitory postsynaptic currents (sIPSCs) and the amplitude of GABA-evoked postsynaptic currents. These endpoints are distinct from those measured for KE682. Therefore, we disagree with the PR in combining KE669 and KE682. |
|  | SR1: this KE is described in term of preceding KEs “a **decline in conductance through chloride channels in iGABARs (another KE)** causes a reduction in GABA mediated inhibition…”. Please only describe “what is neuronal synaptic inhibiton”, and “how is it measured”. Do not describe “what causes it” (i.e. preceeding KEs and KERs). | We revised the section “How This Key Event Works” as suggested by SR1. |
|  | SR2: For "How it works", authors merely state that the previous key event causes a decrease in GABA-mediated signaling and that this decrease can then be measured in a couple of ways. They don’t explain how that happens, although a description of how it happens is included in KE682 EPSP generation. | This is a straightforward event. No additional explanation is required. KE682 is a more complex event that follows KE669. We have made changes to all KEs as suggested by SR1 to remove redundant descriptions overlapping between consecutive KEs. |
|  | SR2: The KE is not described in a way that allows its reuse in other AOPs, because the key event taken by itself implies that the only way to get reduction in neuronal synaptic inhibition is to inhibit the Cl-channels (needs an additional KE between KE682 Cl-channel reduction and KE669 reduced synaptic inhibition).and inhibition.) | This KE occurs as a direct result of decreased chloride channel conductance. Three different MIEs can lead to KE64 and subsequently this KE as we explained above. There is no intermediate process between KE64 and KE669. |
|  | **KE682: Generation, Amplified excitatory postsynaptic potential (EPSP)** |  |
|  | PR: Biologically plausible but difficult to measure. Maybe combine with KE669? | As stated in “How It Is Measured or Detected”, EPSPs can be recorded as [Ca2+] change using intracellular electrodes. A detailed description of the method can be found in Miura et al. (1997). As stated in our response to KE669 comment, this event should not be combined with KE669. |
|  | SR1: The last sentence describes downstream consequences (“produce a sustained increase in hippocampal excitability”), which seems to me like another KE or AO. Please consider removing this last sentence. | The sustained increase in hippocampal excitability is not a downstream consequence but the ending part of KE682. Therefore, we kept the last sentence. |
|  | SR2: The underlying changes that occur to create the integrated cellular response to generate the amplified EPSP actually reveal some additional key events to my mind… inputs from AMPA, NMDA and GABA, alterations of reversal potentials, the integration of EPSP and IPSPs – all of which produce areas for other MIEs to re-use these key events. Then the description describes this as the cause for a sustained increase in hippocampal excitability, which if this tissue level effect is the actual key event you want to describe why not put that in the title? I would recommend splitting this out a bit, perhaps try to separate the cellular-level-effects that you describe in KE682 and KE669 and then create a new tissue level KE of sustained hippocampal excitability if that links better the next KE? Although that might be hard to measure directly at a tissue level, in which case you may just choose to ignore it. | As described in “How This Key Event Works”, GABAA receptor-mediated EPSP plays the pivotal and leading role in epileptogenesis, with the participation of AMPA- and NMDA-mediated EPSPs. Together, these changes in synaptic transmission produce a sustained increase in hippocampal excitability. Almost all biological processes require the consorted action of different players. This KE is initiated by iGABAR-mediated EPSP and involves two other EPSPs, which creates crosslinks to other potential KEs (AMPA- and NMDA-mediated EPSPs of other potential AOP entries. The sustained hippocampal excitation is the manifestation of EPSP at the tissue level, which is an integral part of this KE. So, we can’t separate it from the KE. |
|  | SR2: There are so many underlying processes and levels of biological organization hidden in this KE description that it would be difficult to reuse. | The involvement of other biological processes is required in epileptogeneisis. These crosslinks actually facilitate the reuse of KE682 and other potential KEs (i.e., AMPA- and NMDA-mediated EPSPs) in other AOPs. |
|  | SR2: The level of organization is described at the level of the tissue. Yet tissues don’t have EPSP, individual cells do. So shouldn’t this also be at the cellular level? However, enhanced hippocampal excitability is clearly a tissue description. Splitting these out may help solve these inconsistencies. | We agree with SR2 that EPSP is a cellular level event. We changed to Cellular in “Biological organization” and added “Hippocampal excitation” in “Organ term”. We believe these changes shall clarify the confusion. |
|  | **KE616: Occurrence, A paroxysmal depolarizing shift.** |  |
|  | PR: The event is clearly described and biologically plausible, although mechanisms are not characterized . For use in other AOPs, it might be helpful to the user to cite some open access source(s) rather than text books. Brain location will be important, but perhaps that will be picked up in the external reviews. | The text book we cited (Bromfield et al. 2006) is an open source publication available on NCBI’s website as a web book. We cite it as a source of Evidence Supporting Taxonomic Applicability. |
|  | SR1: This KE is described in terms of “blockage of the ion channel of the iGABAR”. This KE needs to be described **independently** of the MIE and KEs. Biological plausibility and measurement methods OK. | We have accepted SR1 comment and revised the second paragraph by deleting “blockage of the ion channel…” |
|  | SR2: The description of this KE includes a description of the entire adverse outcome pathway, so needs to be edited appropriately. | We have revised section “How This Key Event Works” and eliminated descriptions of upstream and downstream KEs. |
|  | SR2: It seems biologically plausible but I do find one comment particularly confusing: perhaps you could explain how it is that the “subsequent hyperpolarizing afterpotential is mediated by iGABA receptors and Cl influx” if the Cl channel in these receptors is blocked by binding to the picrotoxin site? | As for the mediation of subsequent hyperpolarizing afterpotential by chloride influx, blocking of chloride channel does not mean a complete closure of the chloride channel. There still exists chloride influx (albeit decreased) as well as other influxes such as potassium influx. |
| **AO** | **KE613: Occurrence, Epileptic seizure** |  |
|  | PR: Terminology might hamper use in other AOPs: title says "epileptic seizure", text says "focal epileptic seizures"; is the AO focal partial or generalized epilepsy? | We stated clearly in the second paragraph that focal/partial epileptic seizures would be propagated to the entire brain when there is sufficient activation to recruit surrounding neurons. |
|  | PR: Perhaps check terminology using a recent review e.g. Wendling F, Benquet P, Bartolomei F, Jirsa V. Computational models of epileptiform activity. J Neurosci Methods. 2016 Feb 15;260:233-51. | We appreciate the PR for recommending the review article. However, after glancing through it quickly, we found that it was written for computational modelling and introduced neuroscience terminologies to the computational modeling community. We have adopted and carefully chosen terminologies from original research articles and neuroscience/epilepsy-related reviews. We have strived to use the correct terms and are confident that we have chosen the right technical terms. |
|  | PR: Measurement methods are briefly specified (behavioral, EEG), but no reference. There should be a recent open source summary somewhere… | We accepted this comment and added a reference (Ulate-Campos et al. 2016). We also provided a web link for one to find open source information about how medical doctors diagnose epilepsy in patients. |
|  | PR: Taxonomic applicability cites Tingle et al. (2003) and Gunasekara et al. (2007), both referring to fipronil. Can you find a non-chemical specific reference? | Unfortunately, we have not been able to find a non-chemical specific reference. |
|  | SR1: The AO is described in terms of other KEs (“Blockage of the GABA-gated chloride channel reduces neuronal inhibition and induces focal seizure”, “increase in extracellular K+”, “accumulation of Ca++ in presynaptic terminals”, etc…). Please limit to a more general description of “what is an epileptic seizure?” (what are signs/symptoms, etc…) and “how is it measured”, not “what are the steps that lead up to and induce a seizure”. | We did not intend to cover other KEs in this AO. Instead, we only added these descriptions to lay a good foundation for explaining the complex process of seizure occurrence, symptoms, propagation and progression. |
|  | SR1: The proposed measurement methods need to be better cited. | We added a review paper and a web link as references for measurement/detection/diagnosis methods. |
|  | SR1: The authors indicated that this AO is used by regulators. Are there any relevant references, guidelines or examples? | We have added examples to reinforce the statement about the regulatory application of this AOP. |
|  | SR2: The description of the seizure includes several processes, focal seizure, seizure propagation, seizure termination, generalized seizure, convulsions and death. These seem reasonable and plausible. However, they don’t need to repeat the underling ion changes as they should have been described in the rest of the AOP’s KE. | This comment is similar to those of the PR and SR1. Please see above for our responses above. |
|  | SR2: They mention intercellular effects such as increased extracellular K tending to depolarize neighboring cells, accumulation of presynaptic Ca enhancing neurotransmitter release, and yet these key events are not described elsewhere in the AOP. Perhaps they should be added…… Again the description refers to a lot of the other key events in this AOP and so should be edited to remove those references to allow it to be reused. | Again, we have addressed these comments. Please refer to our responses to the PR and SR1 above. |
|  | SR2: Measurement methods are mentioned but not described or referenced. | We added a review paper and a web link as references. |
| **KERs** | SR1: The KERs and their supporting evidence are often described using up- or downstream KEs that are not specifically relevant to the KER under discussion. | We believe it is necessary to include up- and/or down-stream KEs in order to better describe a KER. Sometimes it is hard to make a convincing case without providing a broader background for the KER under discussion. |
|  | SR1: It is often not very obvious how the presented evidence specifically links KEup to KEdown. Does the presented evidence represent dose-, temporal- or incidence concordance? | In many cases, the KERs are not straightforward due to the complexity of epilepsy, which often involves many players other than those direct participants in the KEs. We presented all available evidence that we could find. Some evidence supports dose-concordance between two KEs, whereas other evidence supports temporal or incidence concordance. |
|  | SR1: It is very important that the authors organize the evidence so that is clear how they demonstrate that changes in KEup are concordant with KEdown. (and only discuss those KEs) | We have tried our best to organize the evidence to demonstrate the concordance and sequential/temporal relationship between KEup and KEdown. |
|  | SR1: Some of this type of information is discussed in the “overall assessment of the AOP”, however it is in the KER sections where specific evidence of these relationships should be presented and cited. | We only presented information relevant to the specific KER. Such information may expand beyond the KEup and the KEdown, but is certainly not intended for overall assessment of the AOP. |
|  | SR2: The KERs again bring in a lot of additional biology that might be expanded into additional key events for a fuller explicit description of the processes. | We have addressed the comment several times in the above. |
|  | SR2: Where a quantitative understanding is available it has been mentioned but not described in detail. | We presented evidence of quantitative KER wherever it is available (e.g., in KER666 and KER667). However, such a quantitative understanding is absent in the other KERs. |
|  | **KER666: Binding at picrotoxin site, iGABAR chloride channel directly leads to Reduction, Ionotropic GABA receptor chloride channel conductance.** |  |
|  | PR: Empirical support reference Akaike et al 1985 is for me unobtainable and from abstract doesn't seem to summarize evidence for Cl- channel effects; can you find a better reference? | We provided two references as empirical support for this KER. Akaike et al. (1985) demonstrated that picrotoxin, applied intracellularly, was capable of blocking GABA-activated chloride current. This constitutes a very strong empirical evidence. We are unaware of any references better than this one. |
|  | PR: Inconsistencies and uncertainties are addressed except for allosteric modulators, which I think should be mentioned here. I'm not sure this is correct: "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." | Allosteric modulators are irrelevant for this KER because they themselves lead to other independent MIEs/AOPs. The statement “There is no known modulator that acts in between receptor binding and channel blocking…” is correct because no factor has been reported that interfere with the relationship between receptor binding and channel blocking. In another word, antagonistic receptor binding directly leads to conformational changes in the iGABAR and results in chloride channel blocking. This is well established. |
|  | **KER683: Reduction, Neuronal synaptic inhibition directly leads to Generation, Amplified excitatory postsynaptic potential (EPSP).** |  |
|  | PR: The description (how it works) overlaps with that for KER2. Again, suggest combining these two. Not sure if the extensive human patient interictal discussion is helpful in this KER- maybe omit? | The PR suggests that the description of “How It Works” overlaps with that of another KER. We cannot identify KER2, nor can we find overlaps between this KER with any other KERs in AOP10.  [Note from PR: Sorry, my error; should have read KER669. See similar comment from SR1 on KER669.] |
|  | PR: Not sure if the extensive human patient interictal discussion is helpful in this KER- maybe omit? | We discussed extensively interictal activity because of its important pathological role in epileptogenesis and in long-term potentiation of synapses. |
|  | **KER684: Generation, Amplified excitatory postsynaptic potential (EPSP) directly leads to Occurrence, A paroxysmal depolarizing shift.** |  |
|  | PR: This KER has multiple biological hypotheses, many modulators, no quantitative understanding of linkage, and there is no information on how to measure. It feels like a KE too many… | Information provided for this KER represents the state-of-the-art knowledge and our interpretation of literature. Based on the mechanistic understanding reflected in literature reports, there exists a clear sequential relationship between the two connecting KEs. However, we also recognize that the quantitative relationship between the two connecting KEs is not yet established, hence we assigned a “moderate” score to the quantitative understanding. |
|  | **KER630: Occurrence, A paroxysmal depolarizing shift (PDS) directly leads to Occurrence, Epileptic seizure.** |  |
|  | PR: "How it works" and weight of evidence are based on Dichter and Ayala (1987) and a brief reference to Jefferys 2010. But isn't PDS just a readout of ongoing epilepsy? Is it really necessary to have it as a KE? Again, maybe a KE too many? | This reviewer questioned if PDS is a readout of epilepsy and if it is necessary to have KE616. Both questions are related to KE616, not KER630. We have addressed the necessity of creating KE682 and KE616 in Section 5. PDS should not be considered as a readout of epilepsy because it is only an intermediate key event of AOP10. |
|  | SR1: Much of the evidence presented is linking interictal discharge (ID) with seizure, but the KER is titled “PDS leads to seizure”. In fact the “empirical support” section makes no mention of “PDS”. The evidence presented must SPECIFICALLY link “KEup: PDS” to “KEdown:Seizures”. It seems as ID and PDS are being presented as the same thing? Please clarify. Additionally, the empirical evidence is limited to one citation from Dichter and Ayala (1987). | The reviewer misunderstood the term “interictal discharge (ID)”. ID is a specific epileptic stage that is characterized by PDS. We introduced and used this term in both the KE and the KER because it is commonly and frequently used in the literature. |
|  | **KER667: Reduction, Ionotropic GABA receptor chloride channel conductance directly leads to Reduction, Neuronal synaptic inhibition.** |  |
|  | PR: Description, plausibility and empirical support OK, except I don't see the relevance of the (Macdonald and Olsen 1994) paragraph here. Maybe add sentence from KER683 "As the dominant charge carrier through GABA-A receptors, chloride is directly implicated in the efficacy of fast neuronal synaptic inhibition (Prescott 2014)". In fact, this sentence could replace the whole text, which is arguably too detailed. | The relevance of MacDonald and Olsen (1994) lies in the fact that it provides direct evidence for picrotoxin and TBPS, both of which cause a decrease in the mean channel open time. We disagree with the PR on adding a sentence from KER683 and replacing the whole text with it. We argue that the sentence only states the role of chloride implicated in the efficacy of fast neuronal synaptic inhibition without providing any empirical evidence. The section referred by the PR here is “Empirical support for linkage”. Hence, the suggestion was not accepted. |
|  | SR1: There is some mention of “seizure induction” which is a downstream AO that is not specifically relevant to this KER. Only include evidence that link the SPECIFIC KEs in this KER. In the “empirical evidence section” there seems to be much discussion about interference with the GABA receptor (an upstream KE?)… but not much evidence SPECIFICALLY LINKING “reduced chloride channel conductance” to “reduced neuronal synaptic inhibition”. Most of the information presented seems to be linking “GABA inhibition” to “reduced CL conductance”, which is the preceding KER … | The SR1 raised concern about including additional and “irrelevant” information. We have addressed this comment several times in the above. No need to reiterate it here. As for the other concern about the lack of specifically linking “reduced chloride channel conductance” with “reduced neuronal synaptic inhibition”, we actually describe the linkage in “Empirical Support for Linkage”. In the 1st paragraph, we introduced the relationship between chloride flux and neuronal synaptic inhibition under the normal condition, whereas in the 2nd paragraph we use picrotoxin as an example of chloride conductance reduction leading to neuronal inhibition reduction. |
|  | **KER684: Generation, Amplified excitatory postsynaptic potential (EPSP) directly leads to Occurrence, A paroxysmal depolarizing shift.** |  |
|  | PR: This KER has multiple biological hypotheses, many modulators, no quantitative understanding of linkage, and there is no information on how to measure. It feels like a KE too many… | Information provided for this KER represents the state-of-the-art knowledge and our interpretation of literature. Based on the mechanistic understanding reflected in literature reports, there exists a clear sequential relationship between the two connecting KEs. However, we also recognize that the quantitative relationship between the two connecting KEs is not yet established, hence we assigned a “moderate” score to the quantitative understanding. |
|  | SR1: The KER is described with reference to “blocked iGABAR channels” (ie the MIE) and PDS (downstream KE). | The reviewer raised again a concern about including additional and “irrelevant” information. We have addressed this comment several times in the above. |
|  | **KER683: Reduction, Neuronal synaptic inhibition leads to Generation, Amplified excitatory postsynaptic potential (EPSP)** |  |
|  | SR1: The KER “reduced GABA mediated postsynaptic inhibition leads to generation of excitatory postsynaptic potential” is indicated as “indirect” in the graphical representation (should be “direct”). | Correction has been made as suggested. |
| **Overall Assessment of the AOP** | PR: Applicability domain: Is this about human epilepsy or mammalian or vertebrate or also invertebrate? Of the cited references, Raymond-Delpech covers only ion channels in insects, Treimann only GABA in human epilepsy. I noticed a paper including the AOP authors which seems equally or more relevant; suggest cite it: Garcia-Reyero N, Habib T, Pirooznia M, Gust KA, Gong P, Warner C, Wilbanks M, Perkins E. Conserved toxic responses across divergent phylogenetic lineages: a meta-analysis of the neurotoxic effects of RDX among multiple species using toxicogenomics. Ecotoxicology. 2011 May;20(3):580-94. | The reviewer may not refer to the Domain of Applicability because we did not cite Raymond-Delpech or Treimann in it. As stated, this AOP is applicable to both vertebrates and invertebrates. We did not cite Garcia-Reyero et al. (2011) in this section because of irrelevance. But we did cite it in the Background section. |
|  | PR: Level of support for essentiality and quantitative understanding of some of the KEs/KERs is a bit vague, arguably because there are too many steps, but also reflecting the literature, which is complex. | We agree with the PR. |
|  | SR1: The support for the essentiality of the KEs is poorly described. Please discuss the essentiality of each KE separately, with specific supporting evidence. | We have discussed the essentiality of each KE in a high degree of details within the description of each KE. We believe it is unnecessary to repeat those details and make a lengthy section.  Follow up comment SR1: The essentiality of KEs for an AOP is a property that is relative to individual AOPs. Therefore, the essentiality CANNOT be described in individual KE descriptions (For example, the proof of essentiality for a single KE would differ with respect to different AOPs). Including the essentiality evidence directly in the KE description would "lock it down" to a single AOP and would prevent the KE being modular to other AOPs. The essentiality of each KE must be substantiated for each individual AOP that it is a part of. Therefore, I find the authors response (that the essentiality is discussed in the individual KE descriptions) not satisfactory. Specific proof of essentiality needs to be presented (experimental, or biological plausibility) for each KE for each AOP it is in. |
|  | SR1: It is unclear how essentiality scores were derived. A transparent discussion of these scores should be presented in the preceding sections. For example, why does KE1 get an essentiality score of 5, whereas KE2 gets a 4? | The methodology for deriving WoE is described in Collier et al. (2016). We presented the results and suggest the SR1 and other interested individuals to read the open-source paper. |
|  | SR2: What is the applicability domain really? The KE clearly describe experimental evidence but the KER descriptions include a lot of descriptions of the underling pathology of epilepsy. I like that the AOP is trying to do that but it strikes me that there is a clear well evidenced AOP from GABA-AR to seizures, convulsions and death in a wide range of species; but the extent to which this has overlapping pathobiology with human clinical epilepsy is a bit more uncertain. | Our intention of developing this AOP is to cover iGABAR (GABAAR) in a wide range of invertebrates and vertebrates. Due to such a broad spectrum of species coverage, we can’t always find the same evidence across different species. Some species such as humans, mice and rats have more evidence than others like earthworms and fishes. We recognize limitations caused by data deficiency and data inequality. |
| **Potential application** | PR: Cites Gong et al (2015) which describes potential applications in more detail. Also says "*Chemicals possessing this AOP* [sic; should be activating this MIE?] *can be distinguished from neurotoxicants acting on other types of iGABAR sites (e.g., orthosteric or allosteric binding sites)*"; however, I am not sure how useful this would be, given that no relevant further information is provided. | We believe that one can easily distinguish chemicals possessing this AOP from other possessing other iGABAR antagonism-based AOPs or other neurotoxicity AOPs when more AOPs become available in the future. AOPs can be used as a chemical classification tool as advocated by OECD. |
| **General Observations and Conclusions** | PR: This is a substantial revision based on a previous internal review (March 2015). The pathway between GABA-A receptor antagonism and epilepsy is plausible and has regulatory relevance. Although I have considerable admiration and sympathy for the authors for tackling such a large literature, the AOP possibly has too many KEs and too much partly relevant detail for the average regulatory toxicologist.  General recommendations regarding future steps of the AOP development: If the points raised by the reviewers are addressed, particularly tighter focus in the descriptions (cf. SR1 below), the AOP could be a potential candidate for external review. | This AOP consists of one MIE, four KEs and one AO, all of which are well defined, characterized and understood. There are many other intermediate events (not key events) and side events involved in the AOP. These events are relevant but some of them are unessential and not well-understood. We presented all of them to paint a detailed picture of the AOP and also to illustrate the complexity of the AOP. |
|  | SR1: Overall, the AOP is biologically plausible and may have regulatory significance. The overall design of the AOP generally fits the recommended format prescribed in the handbook, however, the description and evidence sections need to be written with more focus. KEs are often described in terms of other KEs or the MIE. The KEs must be described as independent measurable events. | We have revised the KEs as suggested. |
|  | SR2: The authors have done a great job assembling the information in the AOP, and present a good case for there being an AOP from GABA-AR binding, inhibition to seizures. |  |
|  | SR1: As with the KEs, the KER descriptions often make reference to up- or downstream KE and KERs. The descriptions should be restricted to the single upstream KE and single downstream KE. It is also not very clear how much of the evidence presented specifically supports the KERs. The authors must clearly indicate how each cited piece of evidence supports the dose-concordance, temporal-concordance or the incidence concordance of the two KEs in the KER (and only those two KEs). | We disagree with the reviewers on the restriction of KERs to only two connecting KEs. We believe that necessary (but limited) expansion of content to relevant events (not necessarily KEs) should be allowed for KER descriptions because in biology, one can’t draw a direct line between two KEs. Many processes are interrelated and cross-linked, forming biological networks (including AOP networks). Without those side events, AOPs won’t be interacted and form connected networks. We should leave room and grant permission for such expansions in KERs. |
|  | SR1: Until the KEs and KERs are described in this fashion [single upstream KE and single downstream KE], they cannot be used as modular components for broader AOP applications. | The modular format required for KEs and KERs and the linear relationship between biological events do not fit the nature of biological processes. Also, an AOP should be considered as a living document that is subject to revision and improvement at future times. Annual or biennial review might be an appropriate mechanism for AOP revisions.  Follow Up Response SR1: The goal of the AOP description (at least to my understanding), and therefore its KE and KER descriptions, is NOT to fully describe the complexity of biological processes, but rather to describe the incidental dependence between biological events/states (i.e. the occurrence of KEupstream has an effect on the probability of KEdownstream occurring). In a practical sense, it does not really matter HOW KEupstream affects KEdownstream, it only matters that it DOES (although “how” CAN be used to establish confidence in the relationship through “biological plausibility”). This sort of relation can almost always (except perhaps in rare instances) be described in a modular, linear fashion. |
|  | SR2: The authors have chosen to chunk up some possible KEs into higher level concepts and the KERs then introduced yet more potential KE that could have been described. I think if they had decided to split out those components of the KE they would have created descriptions with greater potential for reuse in other AOPs and given a more complete description of the pathway for use in computational exploitation of the AOP. However, given the level of detail required I acknowledge that a balance needs to be achieved and it would have made it an even more heroic effort! | We chose very carefully to enlist 4 KEs based on the state-of-the-art and reliable evidence that we summarized from our extensive literature survey. We acknowledge the existence of other potential KEs that may emerge as new knowledge and findings become available. We did not purposely chunk/split or merge any KEs. |
|  | SR2: Should there be a debate about what level of detail (chunking/splitting) in complex AOPs is appropriate in the first instance? | The appropriate level of details (i.e., number of KEs) should be determined by AOP authors based on scientific understanding and strength of technical evidence. |
|  | SR2: The KE descriptions often referred to other KE in their description and this limits their applicability for re-use. | We have revised KEs as required. |
|  | SR2: There is considerable duplication of content between KE and KER descriptions that must have been a considerable effort to write and wonder whether the authors think that the SOAAP/EAGMST should look into whether the way these are described can be streamlined in some way? | We have also noticed this and agree with SR2 that the SOAAP/EAGMST should further modify the template of AOPs to remove obvious redundancy and unnecessary sections or subsections. |