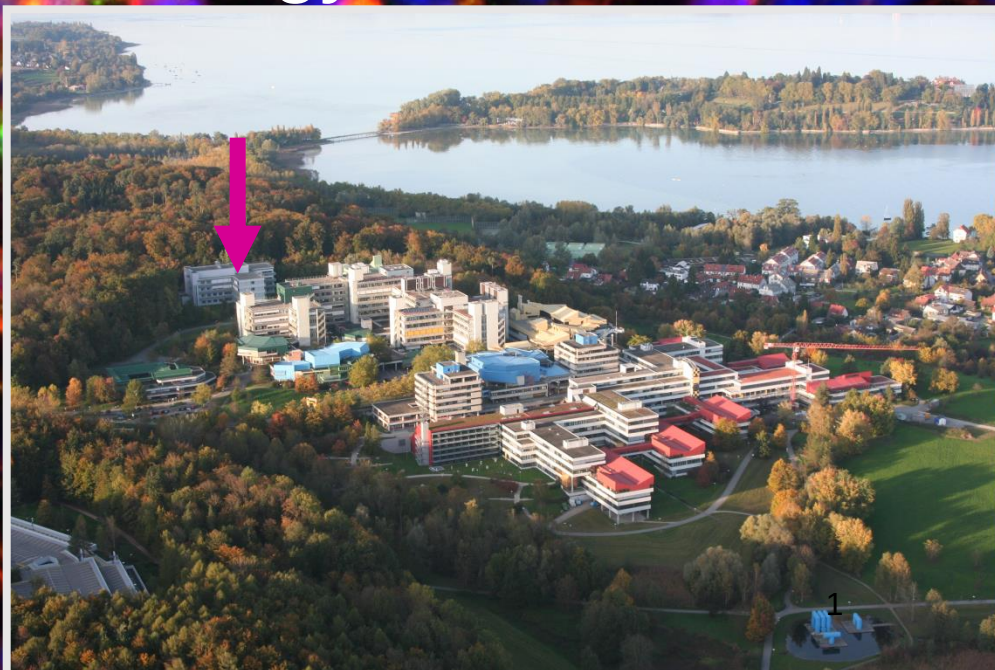
Adverse outcome pathways to bridge the gap between epidemiology and experimental neurotoxicology



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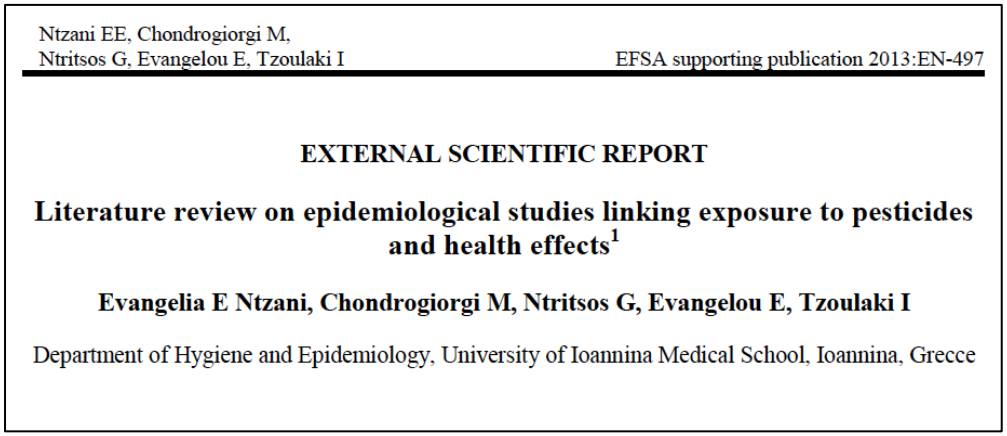
Acknowledgement

EFSA working group
(esp.
Andrea Terron,
Susanne Hougaard,
Anna Price)

Konstanz group
(esp.
Simon Gutbier
Alice Krebs
Johannes Delp)

PESTICIDE EXPOSURE AND HEALTH EFFECTS



Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I — EFSA supporting publication 2013:EN-497

EXTERNAL SCIENTIFIC REPORT

Literature review on epidemiological studies linking exposure to pesticides and health effects[1]

Evangelia E Ntzani, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

Department of Hygiene and Epidemiology, University of Ioannina Medical School, Ioannina, Grecce

OPINION of ANSES
on the INSERM collective expert appraisal report
"Pesticides. Health effects"

An association (statistical correlation) between exposure to pesticides and the risk of developing Parkinson's disease (PD) was found

Are the current toxicity testing methods sufficient to detect adverse outcomes of relevance to human neurological disease, such as PD?

Epidemiological findings linking pesticides to PD

Exposure	Population with a significant excess risk	Presumed link
Pesticides	Professional & non professional	++
Herbicides	Professional & non professional	++
Insecticides	Professional & non professional	++

++: Meta-analyse VAN DER MARK M, BROUWER M, KROMHOUT H, NIJSSEN P, HUSS A, et coll. Is pesticide use related to Parkinson's disease? Some clues to heterogeneity in study results. *Environ Health Perspect 2012, 120 : 340-347*

Chemical classes	Population with a siginificant excess risk	Presumed link
Insecticides Dieldrine	Professional & non professional General population (non smoking)	++ ±
Paraquat	Farmers	+
Rotenone	Farmers	+
Maneb with paraquat	Residential	±

++ Results from several cohort studies
+ Results from one cohort study or 2 case-control studies
± Results from one case-control study

Inserm 2013,

ANSES opinion 2014

→ How do we deal with epidemiology (correlation studies) in pesticide risk assessment?

→ AOP!

Arch Toxicol (2017) 91:3477–3505
DOI 10.1007/s00204-017-2045-3

REGULATORY TOXICOLOGY

Adverse outcome pathways: opportunities, limitations and open questions

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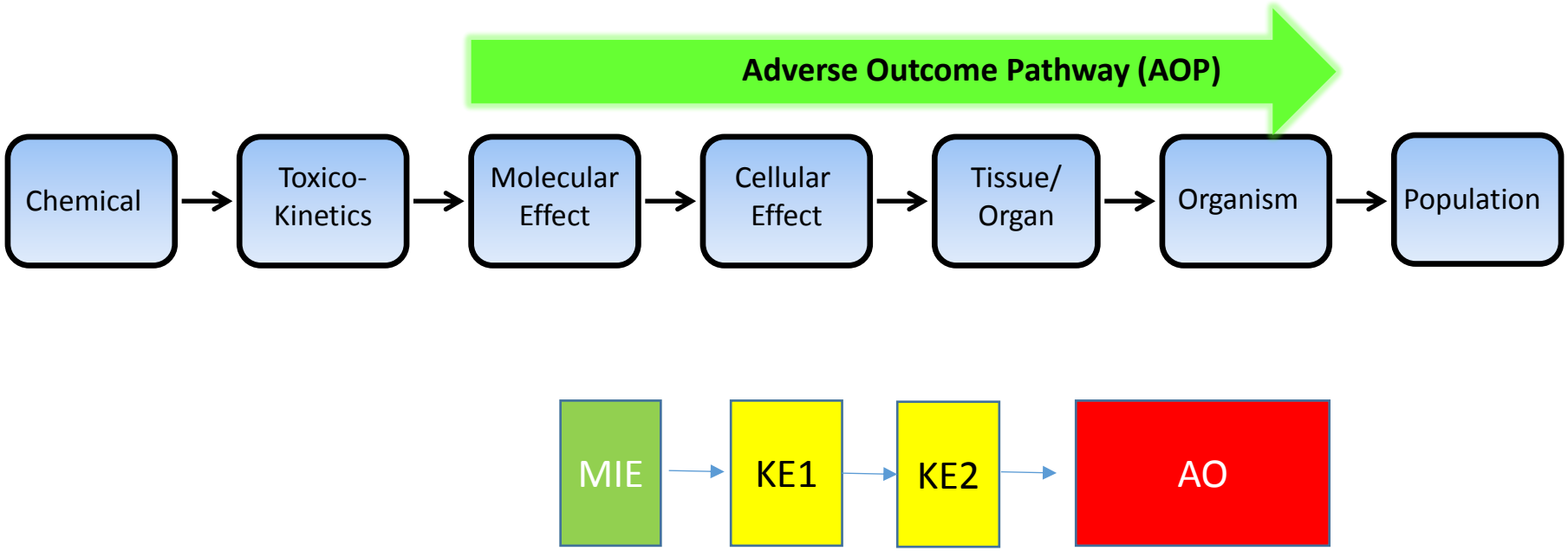
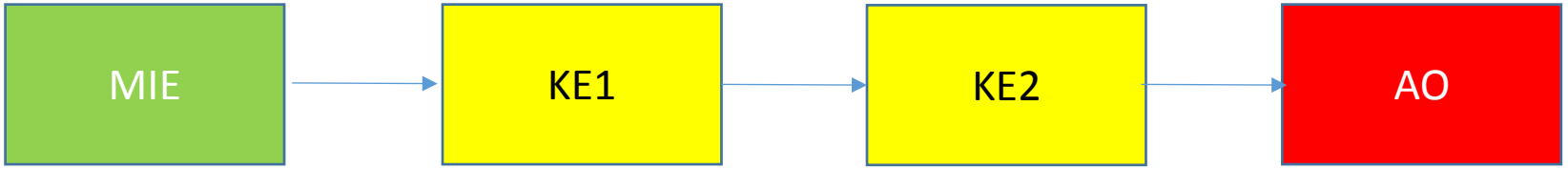
Structuring of a chain of events by AOP



MIE: Molecular initiating event
KE: Key event
AO: Adverse outcome

Construction principles of AOP

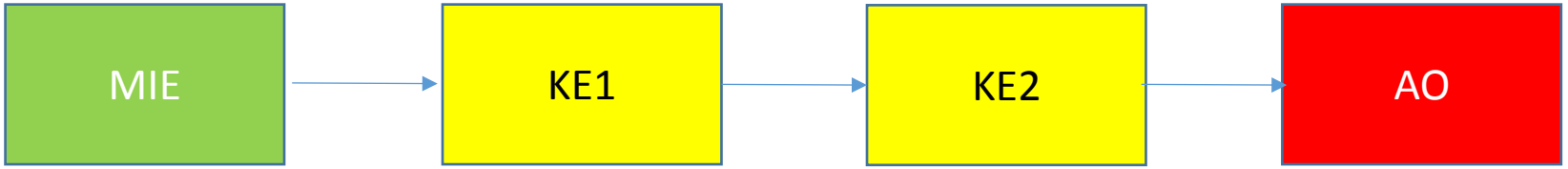


MIE: 1. compound agnostic (**biology-focussed**),
2. preceded by ADME events (local dose relevant for MIE)

An AOP cannot account for ADME/exposure, because this are specific properties of substances

An AOP is characterizing hazard, not risk!!

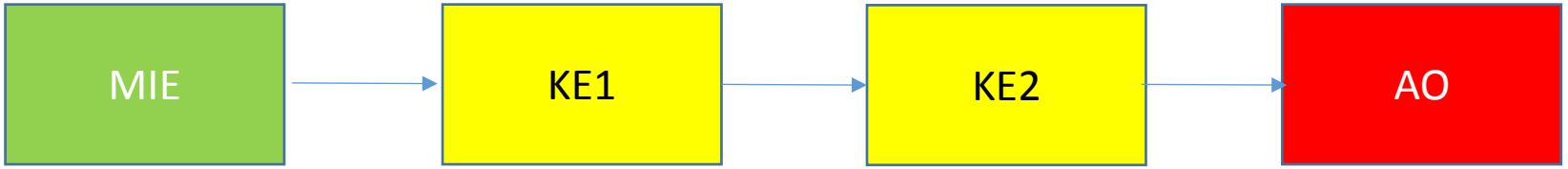
Construction principles of AOP



MIE: compound agnostic (biology-focussed),
preceded by ADME events (local dose relevant for MIE)

KE: must be an **essential** process (necessary, but not sufficient)
has an activation threshold, is **measurable**, is generally observable,
may be shared between AOP

Construction principles of AOP



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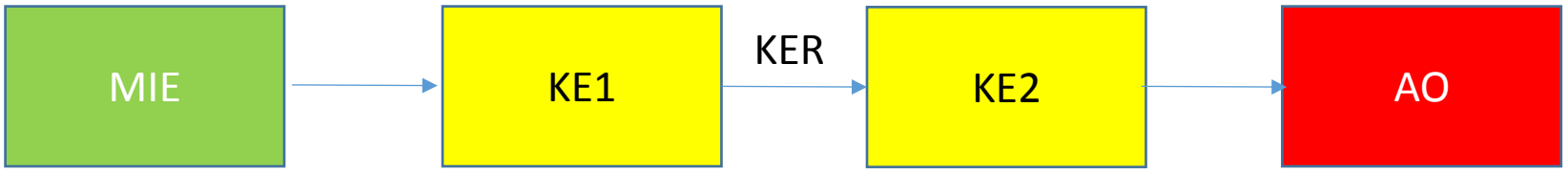

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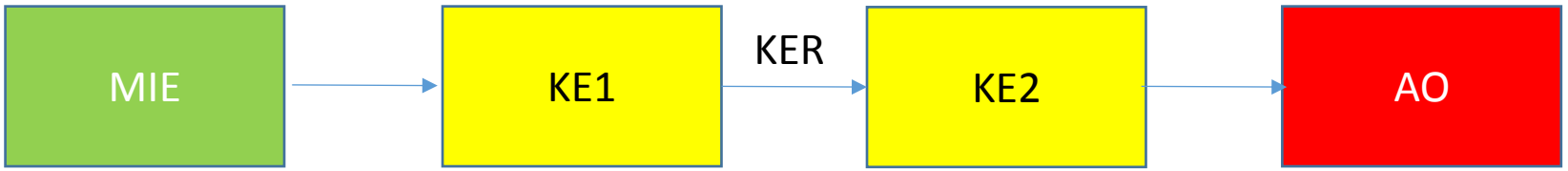
KER: The KE relationship links two blocks. It is AOP specific

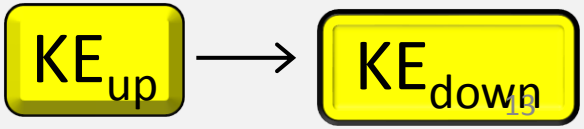
Construction principles of AOP



KER: The KE relationship links two blocks. It is AOP specific

1. How does this KER work?
2. Weight of evidence
   - 2a. Plausibility (biological background knowledge, knock-outs, …)
   - 2b. Correlations/Concordance (in time, dose, etc.)
3. Quantitative understanding of the linkage
4. Uncertainties and inconsistencies
5. Taxonomic applicability

Concordance criterium:
stressors that perturb KE_{up} also perturb KE_{down} in expected fashion, with respect to dose, time and incidence



Features and mis-conceptions of the AOP concept:

1. AOP do not include ADME, and they are compound agnostic
2. **It is a multi-scale data integration tool (sorting and prioritization)**
3. **It can provide plausibility for statistical associations of hazard**
4. **It may be used in risk assessment as element of an IATA**
5. **It can indicate testing deficits and guide testing**

EVENT REPORT

APPROVED: 15 April 2015

PUBLISHED: 20 April 2015

Stakeholder Workshop on the use of Epidemiological data in Pesticide risk assessment

European Food Safety Authority

Exploration of a new strategy (adverse outcome pathway (AOP)-based:

1. **Can AOP make a link of pesticide exposure and human disease plausible?**

2. **Can AOP inform on whether current testing would identify all relevant hazard?**

3. **Can AOP be used to guide improved testing approaches?**

By now, 6 AOP are accepted by ECHA to justify in vitro data submission. Number growing…

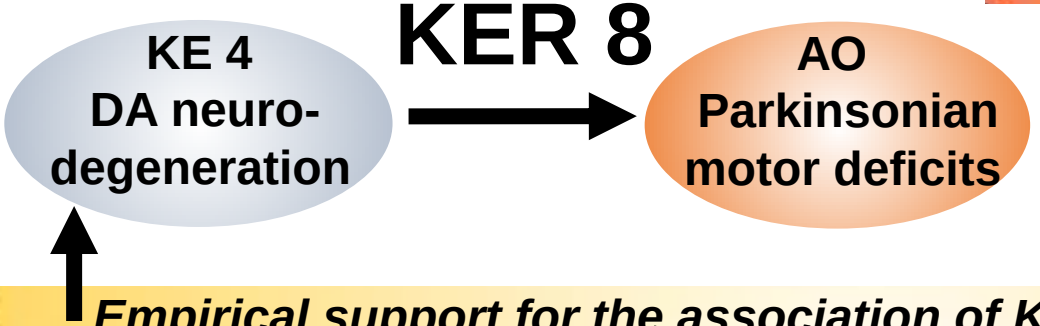
Example of AOP documentation

Biological plausibility/Concordance

- DA neurons of the substantia nigra project into the striatum and release DA [1-3]
- In the striatum, DA is involved in the modulation of motor cortex output as part of the extrapyramidal system [4-6]
- PD is characterized by a decline in striatal DA levels and the onset of parkinsonian motor deficits [7,8]
- Parkinsonian motor deficits are observed at a reduction of striatal DA of > 80 % [9,10]

Uncertainties

- Only limited mechanistic information is available decribing the relationship between the decline in striatal DA and the individual unique PD motor deficits (rigidity, tremor, bradykinesia)
- Degeneration in other brain areas might contribute to the PD phenotype [50-54]
- DAT, VMAT-2, or TH as markers of DA cell loss are problematic due to regulation of expression [55-57]
- Behavioral tests in rodents assess parameters of motor impairment that are not representative for human PD [58]



Weight of Evidence

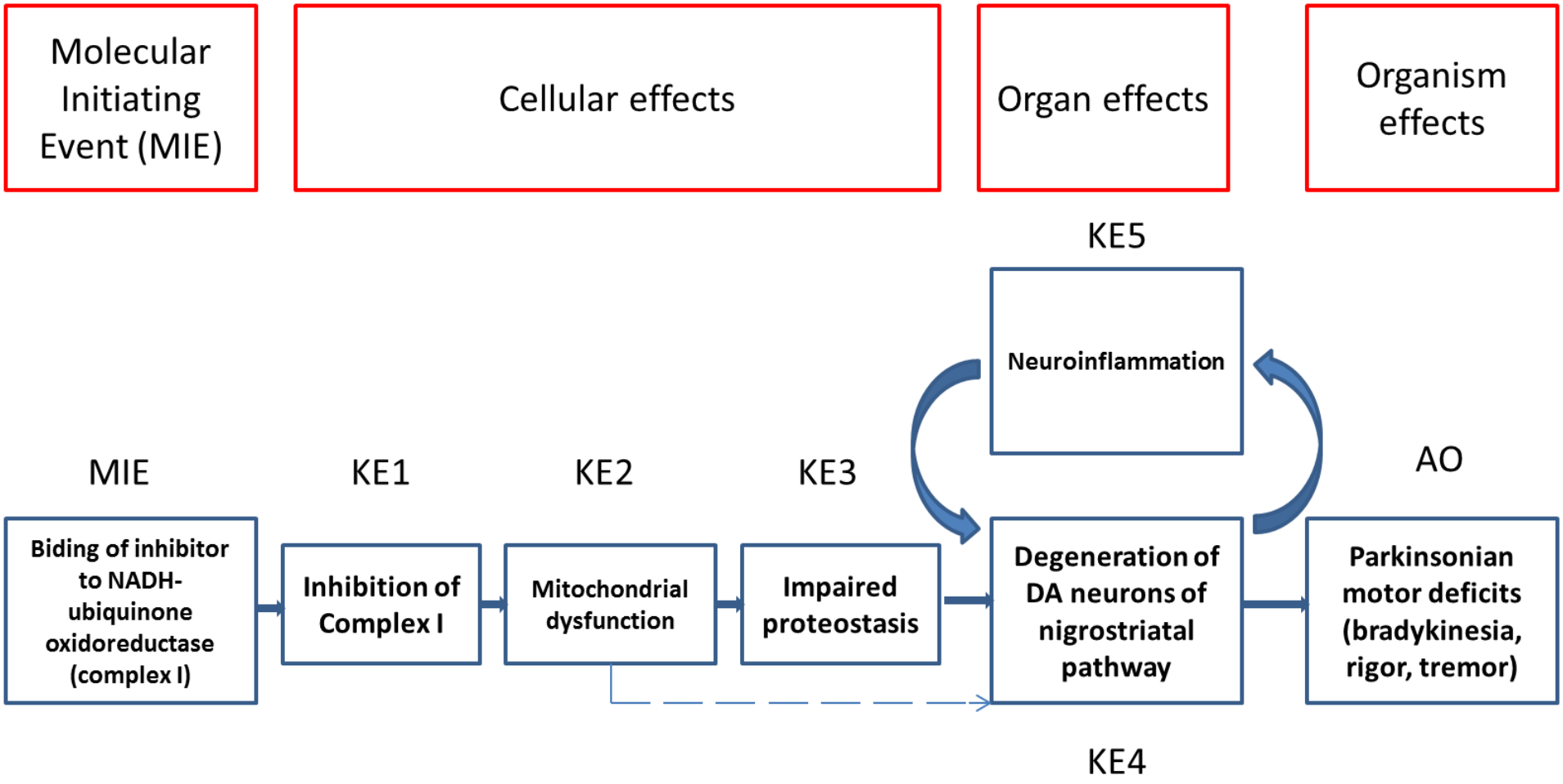
	weak	*moderate*	*strong*
Biological plausibility			X
Empirical support			X

Empirical support for the association of KE 4 with KEs$_{downstream}$

- Analysis of DA levels in post mortem brains and in live PD brains indicates a reduction, directly correlated with the severity of motor deficits [11-18]
- Replacement of endogenous DA (e.g. by L-DOPA) reverses motor deficits [19-30]
- Case studies of tissue grafts or replacement of degenerating DA neurons in the substantia nigra by stem cells indicate a re-innervation of the striatum and an improvement of motor performance [31-36]
- Complex I inhibitor-dependent selective loss of nigrostriatal DA neurons, decline in striatal DA, and the onset of PD motor deficits, as well as its reversal by L-DOPA is constantly observed among humans, non-human primates, and in rodents [37-49]

Terron et al (2018) Arch Toxicol 92, 41-82

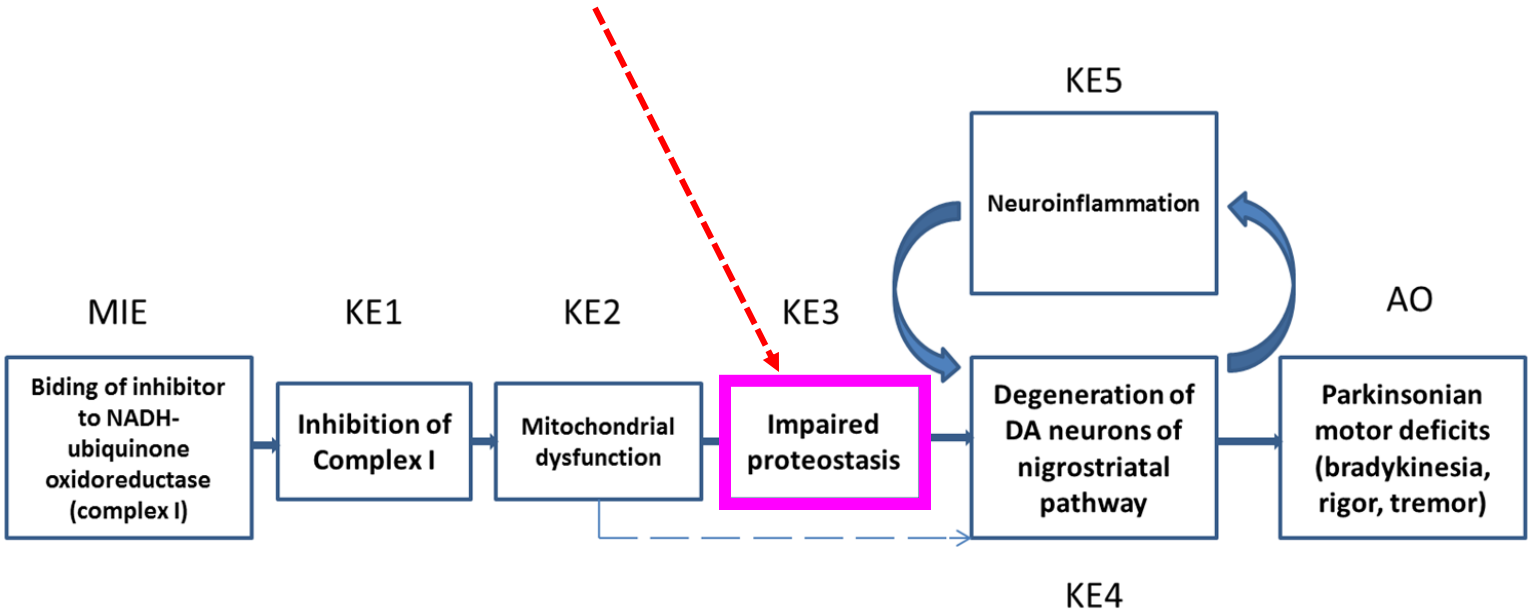
Structuring of a chain of events by AOP



This AOP: Ockleford C et al. (2017) *EFSA J 15, 4691, (325 pages)*
https://aopwiki.org/wiki/index.php/Aop:3

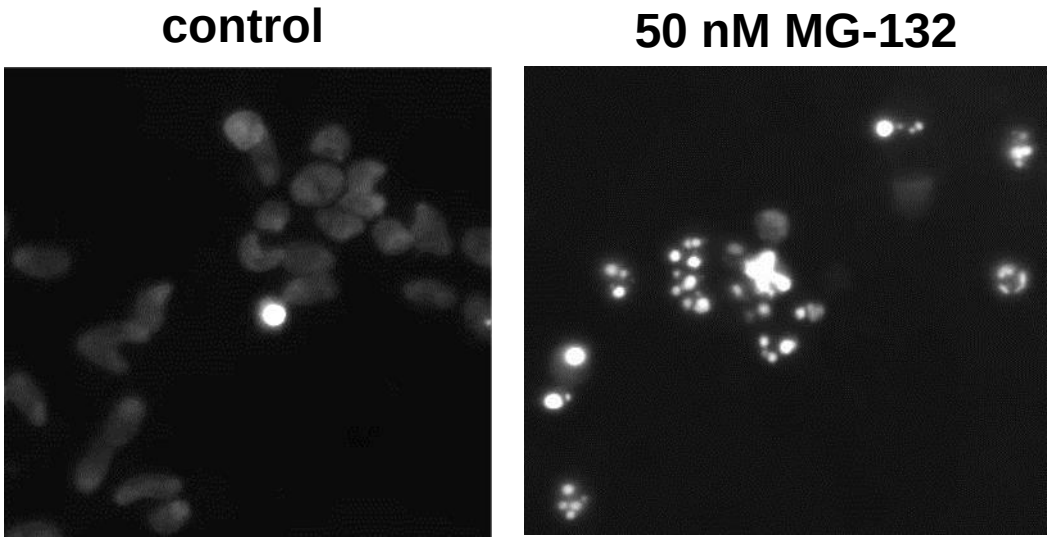
Complex variant: *Schildknecht et al. (2017) TIPS (July issue)*

3rd key event of AOP: impaired proteostasis



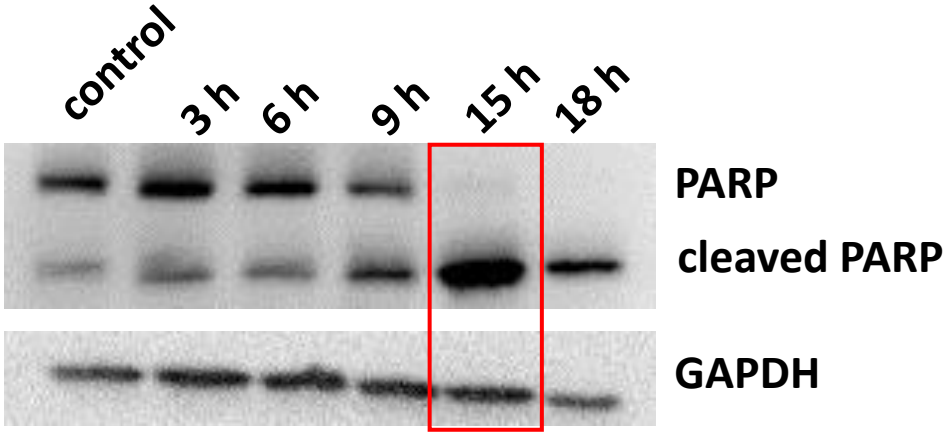
Each of the KE should alone be sufficient to trigger the adverse outcome (e.g. inhibition of proteasome)

The proteasome inhibitor MG132 triggers neuronal death



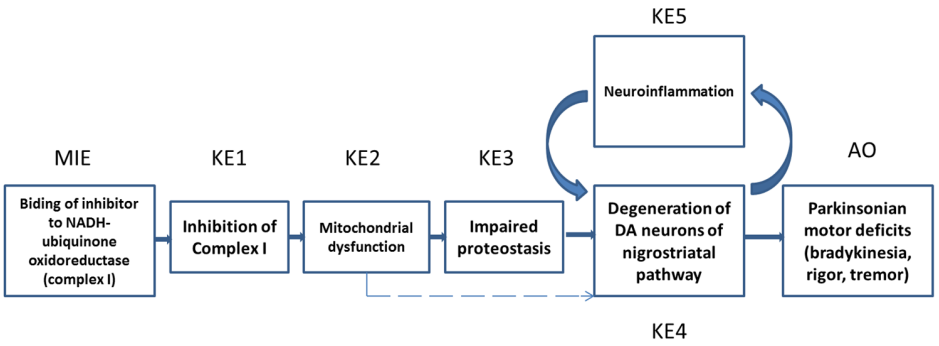
Measurement in vitro (LUHMES human dopamine neurons)

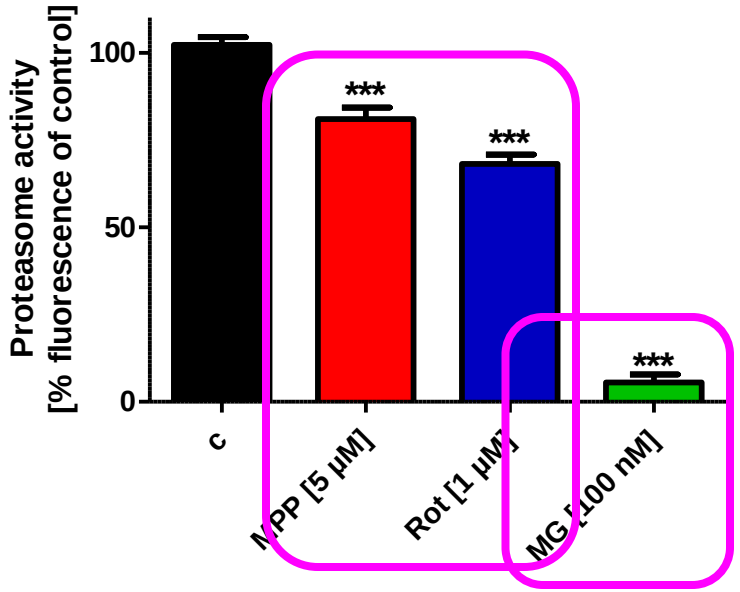
➡ neuronal apoptosis



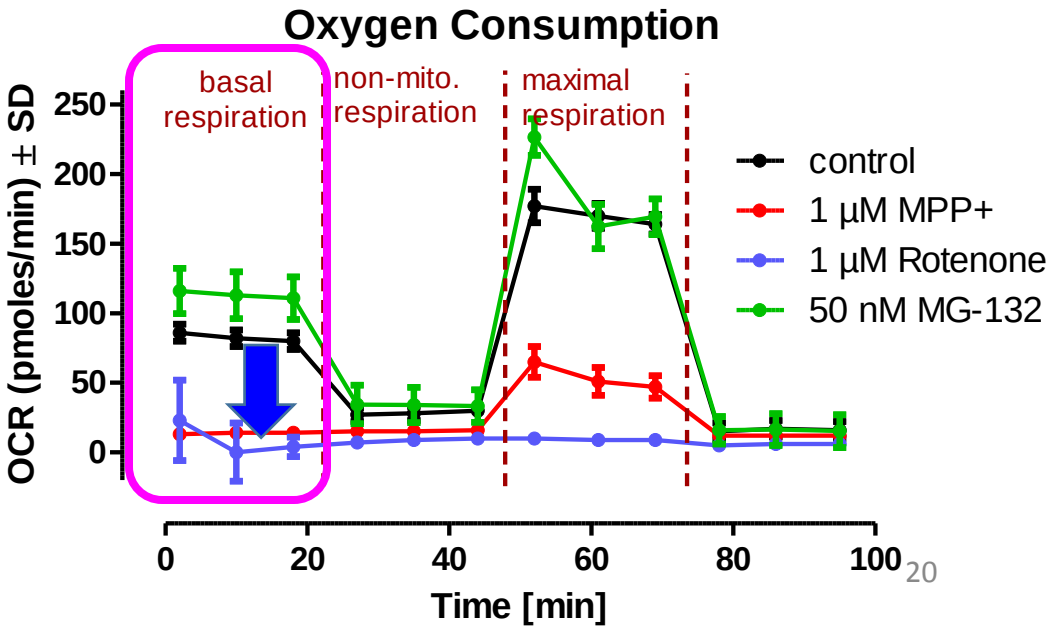
Positioning of proteasome inhibition within the AOP

Rotenone and MPP+ (complex I inhibitors) trigger proteasome dysfunction (KE3)



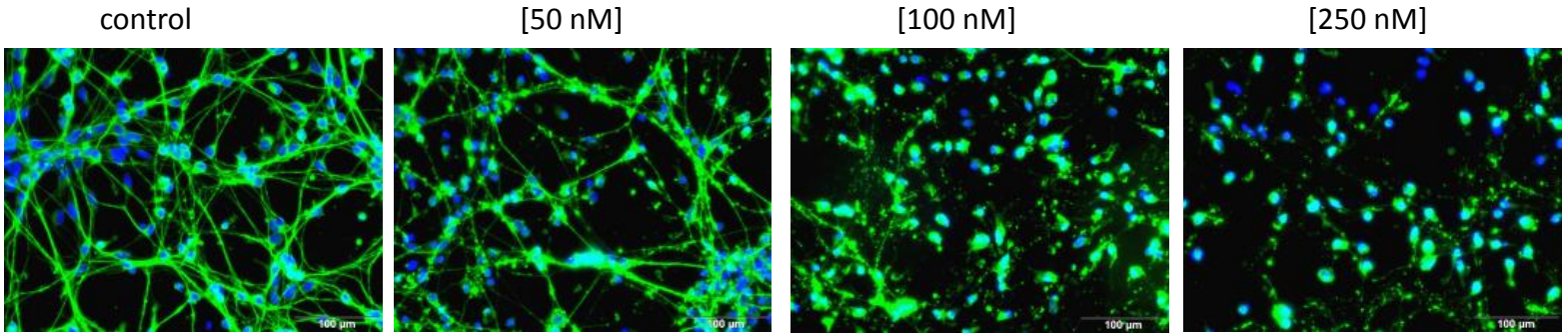


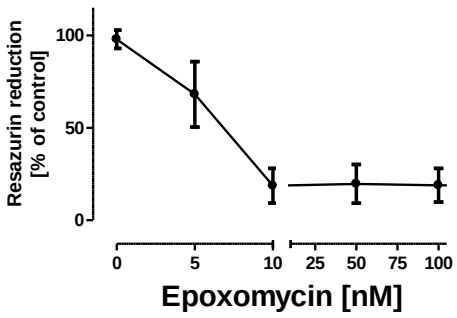
Rotenone and MPP+ block respiration (KE1/2),
but the proteasome inhibitor MG132 (downstream) does not affect respiration

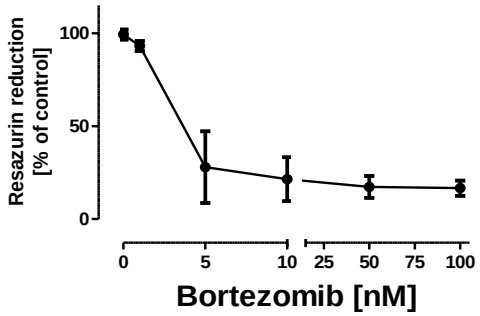


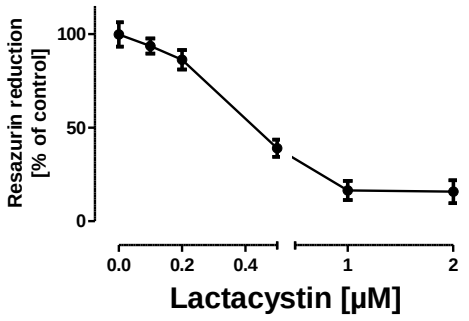
Triggering of degeneration of human neurons by proteasome inhibitors

MG-132



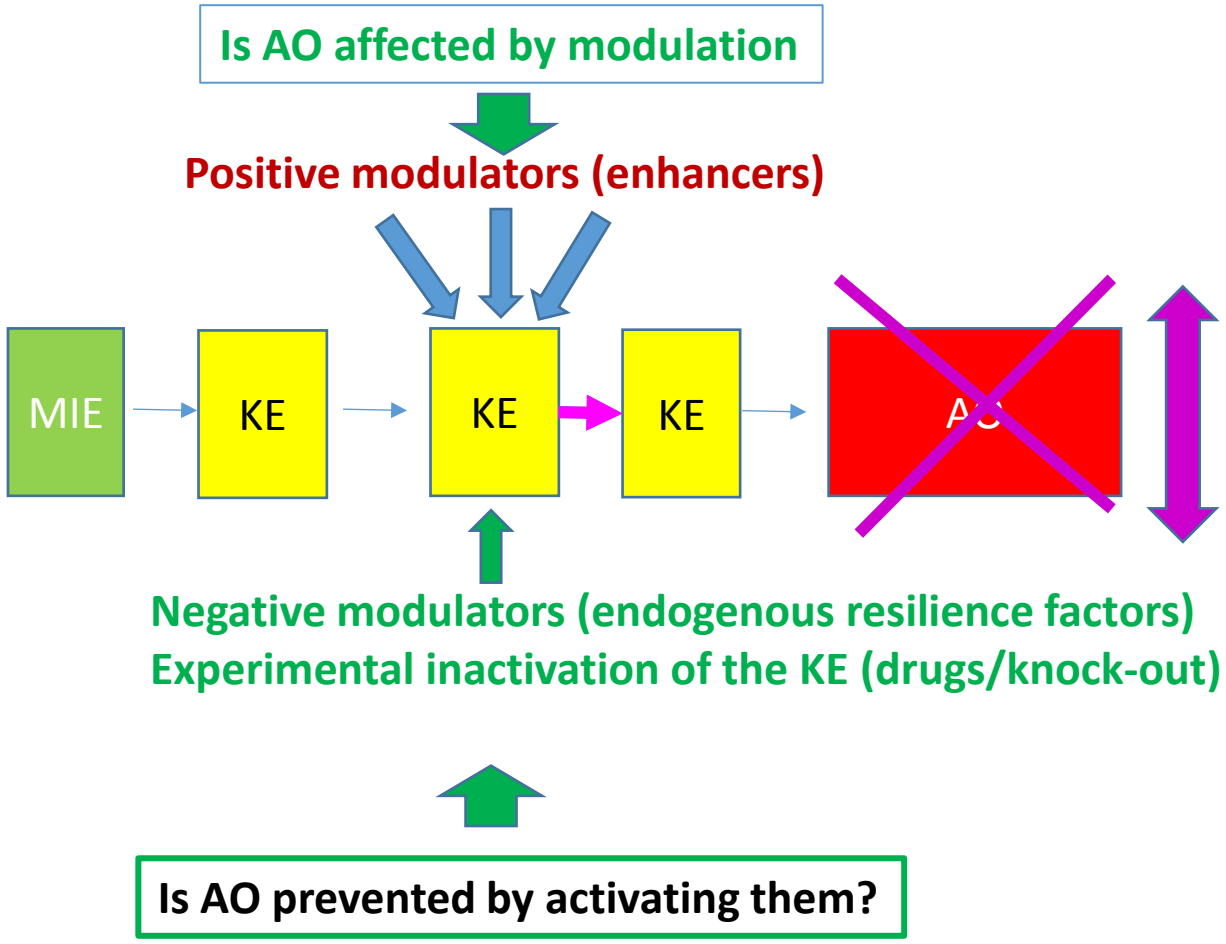




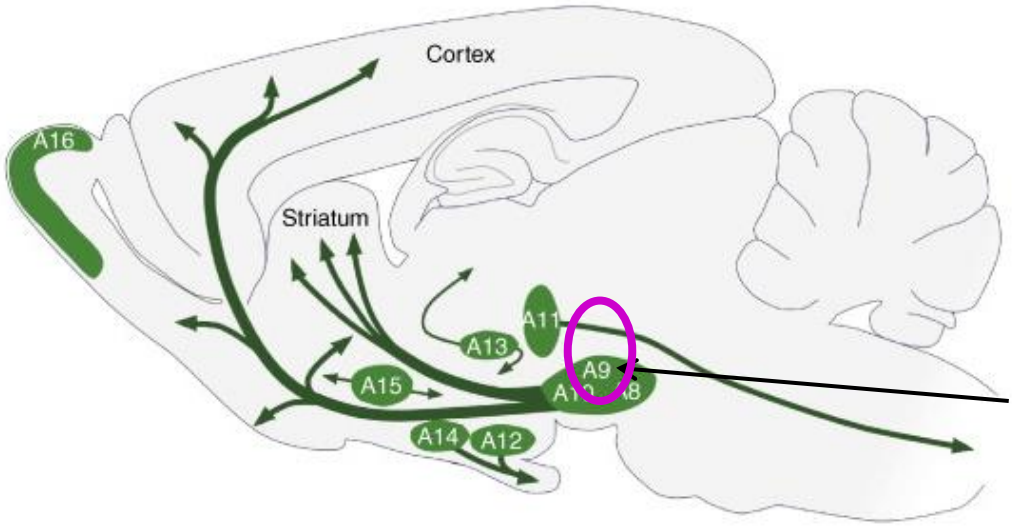


Neurodegeneration triggered by low concentrations of proteasome inhibitors

How to prove essentiality of a KE?

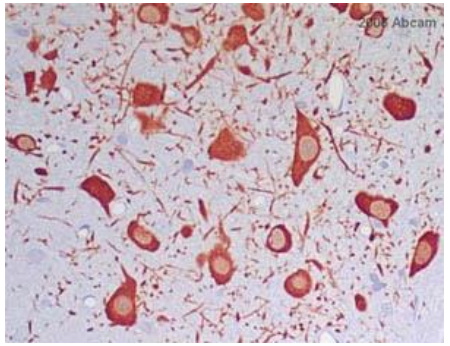


Histopathology of parkinsonian neurodegeneration



Björklund & Dunnet Trends in neuroscience 2007

Loss of dopaminergic neurons of the S. nigra (A9)



IDENTIFICATION OF DA NEURON LOSS IN TOXICOLOGY STUDIES?

- Substantia nigra is in the rostral part of the midbrain – is not investigated in standard studies, but is in neurotoxicity 424 and 426 studies
- Lewy bodies are detected by immunostaining – is not carried out in routine studies
- Only indicator of 'PD' in routine studies is **motor activity** in short term repeat studies
- Only in case of suspected neurotoxicity specialised tests are carried out

Summary of quantitative effects at the concentrations of rotenone and MPTP that trigger the AO (Parkinsonian motor symptoms)

Concentration	KE1 Inhibition of C I	KE2 Mitochondrial dysfunction	KE3 Impaired proteostasis	KE4 Degeneration of DA neurons of nigrostriatal pathway	AO Parkinsonian motor symptoms
Rotenone 20-30 nM rat brain concentration [1, 2, 5, 6]	Approx. 53% [4-5]	Approx. 20-53% (decrease in respiration rate) [1-2]	Approx. 20-60% (decrease in UPS (26S) activity) [3]	Neuronal loss (50% of animal affected) [2]	Motor impairment (100% of animals with neuronal loss) [2]
MPP+ 12-47 µM rat brain concentration [3,4]	Approx. 50-75% [5]	Approx. 38% (reduction in phosphorylating respiration) [5]	Approx. 60% (decrease in UPS activity) [4]	Approx. 50% of neuronal loss [4-5]	Motor impairment [4]

References: Okun et al. 1999 [1]; Barrientos and Moraes 1999 [2]; Borland et al.2008 [3]; Thomas et al 2012 [4]; Betarbet et al 2000 [5] and 2006 [6]

Summary of quantitative effects at the concentrations of rotenone and MPTP that trigger the AO (Parkinsonian motor symptoms)

At concentrations < 20 nM rotenone → neuron loss may be e.g. 40%

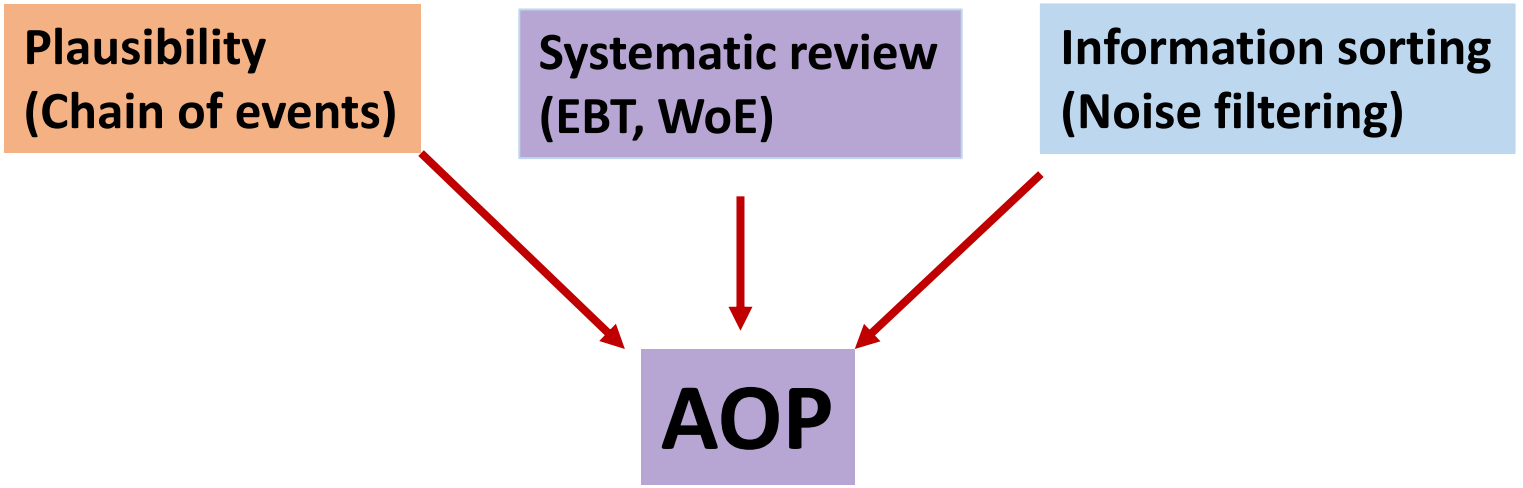
At a neuronal loss of 40% → no motor deficits at all are observed

up to 50% loss: no motor deficit → **not detectable by standard locomotor testing!**

→ **dramatic pathology (40% loss) would remain un-detected by standard toxicological screening**

An AOP-based test battery would provide broader opportunities to detect such toxicity

EFSA conclusions: positive rationale for use of AOP



to structure the information relevant for a potential link of pesticides to PD

to evaluate the suitability of current testing

to guide studies testing for a potential link of pesticides to PD