AOP ID and Title:

AOP 442: Binding to VGSC during development leads to cognitive function decrease
Short Title: Binding to VGSC during development leads to cognitive function decrease

Graphical Representation

Authors

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Abstract

This AOP describes one adverse outcome that may result from the binding of xenobiotics to Voltage Gate Sodium Channels (VGSC) during mammalian development. Binding to VGSC, the molecular-initiating event (MIE; KE1353), results in disruption of sodium channel gate kinetics (KE1977) and consequently to disruption of action potential generation (KE1983); this leads to a subsequent alteration in neurotransmission at all lifestages, but with additional consequences when it occurs during development. Neurotransmitter release is essential for neural activity and neural activity is critical for normal brain development. Disruption of neural activity during development in many brain regions including the hippocampus can negatively impact both neuroanatomy, neurophysiology, and ultimately neurological function. Therefore, chemicals that bind with VGSCs to thwart or augment neurotransmission have the potential to cause adverse effects on the developing brain. When this occurs in the developing hippocampus, it can ultimately lead to impairments in cognitive function. Herein, we discuss the implications of developmental VGSC binding, disruption of action potential generation and neurotransmission during brain development, altered hippocampal anatomy, function, and ultimately higher cognitive processing controlled by the hippocampus. The physiology of VGSC and its essentiality for neurotransmitter release is well known across species. The hippocampus is known to be critically involved in cognitive function, including learning and memory. The adverse consequences of a chemical interference at the VCSC will depend both on severity, duration, and developmental timing, indicating that exposure could produce different effects at different developmental windows of exposure. It is important to note that this could also occur in other areas of the brain as VGSC are foundational to the structure and function of all neurons. Here we focus on the hippocampus because of its well-known ties to cognition, and downstream outcome of concern for many chemical exposures, but there is less empirical evidence and biological knowledge on the adverse consequences in other brain areas, The overall weight of evidence for this AOP is strong. Gaps in our understanding include the specific critical developmental windows and the quantitative relationship of binding to VGSC and subsequent disruption and cognitive function. Although quantitative information at all levels of KERs is limited, a number of regulatory applications of this AOP for DNT assessment have been identified.

Summary of the AOP

Events

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

Sequence	Type	Event ID	Title	Short name
1	MIE	1353	Binding to voltage-gated sodium channel	Binding to VGSC
2	KE	1977	Disruption of sodium channel gating kinetics	Altered kinetics of sodium channel
3	KE	1983	Disruption, action potential	Disruption in action potential generation
4	KE	2005	Altered neurotransmission in development	neurotrasmission in development
5	KE	757	Hippocampal anatomy, Altered	Hippocampal anatomy, Altered
	KE	758	Hippocampal Physiology, Altered	Hippocampal Physiology, Altered
	AO	402	Cognitive Function, Decreased	Cognitive Function, Decreased

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Binding to voltage-gated sodium channel	adjacent	Disruption of sodium channel gating kinetics		
Disruption of sodium channel gating kinetics	adjacent	Disruption, action potential		
Disruption, action potential	adjacent	Altered neurotransmission in development		
Altered neurotransmission in development	adjacent	Hippocampal anatomy, Altered		
Hippocampal anatomy, Altered	adjacent	Hippocampal Physiology, Altered		
Hippocampal Physiology, Altered	adjacent	Cognitive Function, Decreased		

Stressors

Name	Evidence
Pyrethrins and Pyrethroids	High

Pyrethrins and Pyrethroids

Natural toxins, produced by animal, plant and microorganisms, target VGSCs through diverse strategies developed over millions of years of evolution. The sodium transients can be antagonised by TTX (tetrodotoxin) (Káradóttir et al., 2008; Berrett et al., 2017) which is the classic stressor. Classic and well studied stressors for VGSCs are pyrethroid insecticides. Indeed, it is well known and accepted that pyrethroids bind to the α subunit of the neuronal VGSC (Trainer et al., 1997; Smith and Soderlund, 1998, 2001; Catterall et al., 2007; Cao et al., 2011). Mutations in the α subunit of both insects (Lee and Soderlund, 2001; Smith et al., 1997) and mammals (Vais et al., 2000, 2001; Wang et al., 2001) alter the sensitivity of VGSCs to pyrethroids, supporting the conclusion that pyrethroid interact with the α subunit (Shafer et al., 2005). The β subunit has been observed to modulate the affinity of pyrethroid interaction with the channel (Smith and Soderlund, 1998). However, the pyrethroid sensitivity of VGSCs subunits and splice variants expressed during development has yet to be examined (Shafer et al., 2005). The actions of pyrethroid insecticides on sodium channels in invertebrate and vertebrate nerve preparation have been widely documented over the past decades and has been extensively and critically summarised in numerous reviews (Soderlund et al., 2002; Chahine, 2018). Based on their chemical structure and clinical symptoms of toxicity, pyrethroids are classified in type I and type II. Following the binding to a VGSC specific isoform/s, pyrethroid slow the activation or opening, of VGSC. In addition, they slow the rate of VGSC inactivation (or closing) and shift to a more hyperpolarised potentials the membrane potentials at which VGSC activate (or open) (Narahashi, 1996). The result is that sodium channels open at more hyperpolarised potential and are held open longer, allowing more sodium ions to cross and depolarise the neuronal membrane. Type II pyrethroids delay the inactivation of VGSCs longer than do type I pyrethroids, leading to a depolarisation-dependent block. These differences in prolongation of channel open times are considered to contribute to the different toxicological profile (Ray 2001).

Overall Assessment of the AOP

Determination of confidence in the overall AOP as a basis to support specific regulatory application relies on the biological plausibility, empirical support, and quantitative understanding of the KERs, as well as the evidence supporting essentiality of the KEs. Table 1 provides an overall summary of the weight of evidence based on the evaluations of the individual linkages from the Key Event Relationship pages. It indicates how biological plausibility and empirical evidence improved with the new work reported here (e.g. from moderate to strong of biological plausibility of KER749.

Please, refer to Appendix B1. Statistical Analysis report for the description of the methodology and individual assessment in the Expert Knowledge Elicitation.

Table 1. Summary table with the assessment of the relative level of confidence in the overall AOP based on rank ordered weight of evidence elements and Expert Knowledge Elicitation.

	KER2605 Direct KER	**KER2625** Direct KER	**KER3242** Direct KER	**KER3243** Direct KER	**KER749 with new data** Direct KER	**KER748 with new data** Direct KER
Biological Plausibility	**STRONG**	**STRONG**	**STRONG**	**STRONG**	From moderate to **STRONG**	From moderate to **STRONG**
Empirical Evidence	**STRONG**	**STRONG**	**MODERATE**	**MODERATE**	From moderate to **MODERATE-STRONG**	From moderate to **STRONG**

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
During development and at adulthood	

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Rattus norvegicus	Rattus norvegicus	High	NCBI
mouse	Mus musculus	High	NCBI
humans	Homo sapiens	High	NCBI

Sex Applicability

Sex	Evidence
Male	High
Female	High

Chemicals: This AOP applies to a wide range of chemicals that binds to VGSC. Well recognized prototypical stressors include natural toxins, TTX (classic stressor), pyrethrins and pyrethroids.

Pyrethrins and Pyrethroids

Natural toxins, produced by animal, plant and microorganisms, target VGSCs through diverse strategies developed over millions of years of evolution. The sodium transients can be antagonised by TTX (tetrodotoxin) (Káradóttir et al., 2008; Berrett et al., 2017) which is the classic stressor. Classic and well studied stressors for VGSCs are pyrethroid insecticides. Indeed, it is well known and accepted that pyrethroids bind to the α subunit of the neuronal VGSC (Trainer et al., 1997; Smith et al., 1997; Smith and Soderlund, 1998, 2001; Catterall et al., 2007; Cao et al., 2011). Mutations in the α subunit of both insects (Lee and Soderlund, 2001; Smith et al., 1997) and mammals (Vais et al., 2000, 2001; Wang et al., 2001) alter the sensitivity of VGSCs to pyrethroids, supporting the conclusion that pyrethroid interact with the α subunit (Shafer et al., 2005). The β subunit has been observed to modulate the affinity of pyrethroid interaction with the channel (Smith and Soderlund, 1998). Further work indicates that deltamethrin effects on sodium currents were dependent on subunit-combinations and the embryonically expressed Nav1.3/β3 channels were more sensitive than the Nav1.2/β1 channels expressed in adulthood. Moreover, the Nav1.3/β3 channels were particularly sensitive to cyano-containing pyrethroids (type II pyrethroids, e.g., cypermethrin, β-cyfluthrin, esfenvalerate and fenpropathrin) but not for the type I pyrethroids permethrin and tetramethrin (Meacham et al. 2008). Additional studies demonstrated that orthologous channels with a high degree of amino acid sequence conservation differ in both their functional properties and their sensitivity to pyrethroid insecticides. Thus, e.g. human Nav1.3 channels are not only less sensitive than the rat Nav1.3 channels but also less sensitive than the relatively less sensitive rat Nav1.2 channels (Tan and Soderlund, 2009, Bal-Price et.al., 2008).

However, the action of pyrethroid insecticides on sodium channels in invertebrate and vertebrate nerve preparation has been widely documented over the past decades and extensively and critically summarised in numerous reviews (Soderlund et al., 2002; Chahine, 2018). Based on their chemical structure and clinical symptoms of toxicity, pyrethroids are classified in type I and type II. Following the binding to a VGSC specific isoform/s, pyrethroids slow down the activation (or opening), of VGSC. In addition, they reduce the rate of VGSC inactivation (or closing) and shift to the membrane potentials at which VGSC activate (or open) to a more hyperpolarised state (Narahashi, 1996). As a result, sodium channels open at more hyperpolarised potential and remain open for longer, allowing an increased influx of sodium ions that can eventually depolarise the neuronal membrane. Type II pyrethroidsprolong VGSCs inactivation more than type I pyrethroids, leading to a depolarisation-dependent block. These differences in channel open times contribute to the distinct toxicological profiles of these chemicals (Verschoyle and Aldridge 1980 Ray, 2001). See Figure 5 below from Shafer et al. (2005).

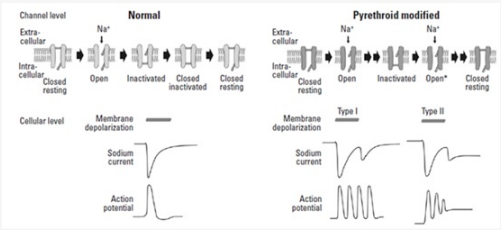

Figure 5: Pyrethroid effects on neuronal excitability. Pyrethroids inhibit the function of 'gates' that control sodium flux through VGSC, delaying inactivation (indicated by the double arrow between states) of the channel and allowing continued sodium flux. After depolarisation ends, pyrethroid-mediated VGSC remain open, resulting in a 'tail' current. Type I pyrethroids action results in a series of action potentials, while type II pyrethroids cause greater membrane depolarisation, leading to a depolarisation-dependent block. Source: Shafer et al., 2005.

Figure 5 summarises the effects of pyrethroids on individual channels, whole-cell sodium currents and action potentials.

Essentiality of the Key Events

In accordance with the OECD AOP Handbook the essentiality addresses the impact of manipulation of a given KE on the downstream sequence of KEs defined for the AOP.

It is noted that for this AOP it is widely accepted that each of the key events is essential. In addition, a large amount of publications using knock-out methods were retrieved for the different KEs. Although they do not provide conclusive evidence on essentiality they have been compiled and used for both essentiality and biological plausibility of the KERs (see **Table 3** in Appendix E).

The mutation studies addressing the KERs within this AOP have been carried out both in vitro and in vivo. Specifically, for KER4 these types of studies have been conducted in knockout mouse models. These studies combine electrophysiological analyses of acute brain slices with methods (e.g., immunohistochemistry) to characterize role of knockout proteins in hippocampal function. Since KE3 and KE4 are usually measured in the same study, it is difficult to determine which one occurs first (KEup) and which occurs later (KEdown). As a result, no firm conclusion can be drawn on essentiality, but this evidence is considered proof of it.

Weight of Evidence Summary

Biological Plausibility

		Defining Question	**High (Strong)**	**Moderate**	**Low (Weak)**
1.Support for Biological Plausibility of KERS	**Direct or Indirect KER**	Is there a mechanistic relationship between KEup and KEdown consistent with established biological knowledge?	Extensive understanding of the KER based on extensive previous documentation and broad acceptance.	KER is plausible based on analogy to accepted biological relationships, but scientific understanding is incomplete	Empirical support for association between KEs, but the structural or functional relationship between them is not understood.

KER1: KER2605 Binding to voltage-gated sodium channel leads to Altered kinetics of sodium channel	Direct	The biological plausibility for this KER is **strong.** It is a well-accepted fact that ion channels are integral membrane proteins that control the passage of various ions (Cl−) across lipid membranes in cells. The direction of ion transport through an open ion channel is governed by the electrochemical gradient for the particular ion sp membrane in question. There is overwhelming evidence that binding of a chemical to a VGSC alters sodium channels kinetics. This is well supported by studies in wh channel residues are mutated, and these mutations alter the ability of different chemicals to interact with the sodium channel to alter its gating kinetics (e.g. Vais et 2001). The stereospecific nature of effects of many different compounds on VGSC function further supports that specific binding leads to alterations in the kinetics of (Soderlund 1985; Brown et al., 1988; Narahashi 1982).
KER2: KER2625 Altered kinetics of sodium channel leads to Disruption in action potential generation	Direct	The biological plausibility of KER2 (Altered kinetics of sodium channel leads to Disruption in action potential generation) is **strong**. The rising phase of an action pote by the opening of voltage-gated sodium channels. These ion channels are activated once the cell's membrane potential reaches a threshold and open immediately. T electrochemical gradients drive sodium into the cell causing a strong and abrupt depolarization characteristic of an action potential. The falling phase of the action p caused by the inactivation of the VGSCs stopping further sodium influx, and the opening of voltage-gated potassium channels. As K+ concentrations inside the cell a channels open and the current flow out serves to restore the membrane potential toward its resting state. However, the efflux of K+ ions is large, leading to a hyperp (undershoot phase) of the membrane potential. Ultimately the voltage-gated K+ channels close and the membrane potential returns to its resting state. This is very textbook knowledge. While it is well accepted that various combinations of channel types in a cell can give rise to differences in the shape and time course of the act the underlying biological principles and relationships between VGSC and action potentials are maintained. Expression of VGSCs is spatially and temporally dependen differential expression during CNS development. It is clear that as in the adult, binding to VGSC isoforms will also disrupt the channel gating kinetics and action pote in the developing brain (see reviews by Shafer et al., 2005; Soderlund et al., 2002).
KER3: KER3242 Disruption of action potential leads to altered neurotransmission during development.	Direct	The process of disruption of action potentials leading to changes in neurotransmission represents a very well-established principle of neurobiology that is described in the published literature and basic neuroscience textbooks. The biological plausibility is **strong**. This process is the basis of routine neurophysiol investigating the development, function and disturbance of neuronal networks. It is not only biologically plausible that alterations in action potential shape, duration could lead to altered neurotransmission, but also that this occurs in adult and developing nervous systems.
KER4: KER3243 Altered neurotransmission during development leads to altered hippocampal anatomy	Direct	The biological plausibility of altered neurotransmission during the development and further impairment of hippocampal anatomy is **strong.** Extensive evide the notion that disruption of neurotransmission during development can induce micro-structural morphological changes in the hippocampus. This can occur due to th various factors such as genetic mutations, brain damage, environmental toxins, and stress during vulnerable periods of brain development. Impaired synaptic transmission may occur at pre- or postsynaptic level and involves disruption of the normal functioning of neurotransmitters, their receptors, or sca The strength of the synaptic transmission can be modulated by the amount of neurotransmitter released, the number of receptors on the postsynaptic cell, and their the neurotransmitter due to alterations in the number and conductance of postsynaptic receptors (Graziane and Dong, 2022; Hestrin, 2015). In case of presynaptic d either too much or too little neurotransmitter may be released into the synaptic cleft, whereas in postsynaptic dysfunction, the postsynaptic neuron may not respond that neurotransmitter. In both cases, the altered synaptic transmission may have pre- or postsynaptic morphological consequences, including e.g. number of docked nerve terminal, or the number, density and morphology of dendrite spines. These changes may affect the structure and function of neural circuits and may underlie deficits (Bonnycastle et al., 2021).
KER5: KER749 Hippocampal anatomy altered leads to hippocampal physiology altered	Direct	The biological plausibility of alterations in hippocampal structure impacting synaptic function and plasticity in the brain is **strong**. Because synaptic transmission in t relies on the integrity of contacts and the reliability of electrical and chemical transmission between pre- and post-synaptic neurons, it is well accepted that interfere anatomical levels will largely impact the functional output on the neurophysiological level (Knowles, 1992; Schultz and Engelhardt, 2014). Extensive research has provided substantial data on the characteristics supporting a direct link between alterations in neuronal anatomy (axon and dendritic spines shape and density, vesicular proteins and release, synaptogenesis and neuronal network formation) and neurotransmission, particularly in the context of activity-dep changes in synaptic strength (synaptic plasticity), best exemplified in the phenomenon of long-term potentiation (LTP). For instance, spine structure is closely linked function, as the size of spine heads scales with synaptic strength (Matsuzaki et al., 2001; Noguchi et al., 2011). Moreover, the shape and number of spines can be m induction of synaptic plasticity (Matsuzaki et al., 2001; Tønnesen et al., 2014; Zhou et al., 2004). These anatomical alterations in hippocampus lead to changes in th electrophysiological properties of this brain region. Specifically, they serve as physiological readouts of hippocampal function at the synaptic level. The most commo readouts were revealed as impairments in basal neurotransmission, synaptic inhibition, and synaptic plasticity (LTP and LTD) (Schnell et al.,2002; Ehrlich & Malinow, Malinow, 2004; Schmeisser et al., 20129). As detailed in KER4, these same activity-dependent processes are invoked as mechanistic underpinnings for how neuronal structure, especially in the developing brain.
KER6: KER748 Hippocampal Physiology Altered leads to Cognitive Function Decreased	Direct	The biological plausibility of the KER is rated as **strong**. It is well accepted that the normal hippocampal function is critical for the acquisition and memory of cont spatially mediated tasks in rodents and humans (Sweatt, 2016).

Empirical Support

		Defining Question	**High (Strong)**	**Moderate**	**Low (Weak)**
2. Empirical Support for KERs	**Direct or Indirect KER**	Does empirical evidence support that a change in KEup leads to an appropriate change in KEdown? Does KEup occur at lower doses and earlier time points than KE down and is the incidence of KEup > than that for KEdown? Inconsistencies	Multiple studies showing dependent change in both events following exposure to a wide range of specific stressors. No or few critical data gaps or conflicting data	Demonstrated dependent change in both events following exposure to a small number of stressors. Some inconsistencies with expected pattern that can be explained by various factors.	Limited or no studies reporting dependent change in both events following exposure to a specific stressor; and/or significant inconsistencies in empirical support across taxa and species that don't align with hypothesized AOP
KER1: KER2605 Binding to voltage-gated sodium channel leads to Altered kinetics of sodium channel	Direct	The empirical evidence for this KER is **STRONG**. A wide variety of natural-occurring toxins have been demonstrated to interact with VGSCs and alter function of the c the poison in fugu (pufferfish); scorpion and sea anemone toxins, brevotoxins from dinoflagellates, ciguatoxins and some conotoxins from fish-hunting snails. VGSCs receptor sites on the VGSC protein, and binding to each of these sites has differential effects on channel kinetics. For example, TTX binds at site 1, irreversibly blocki preventing sodium from moving through the channel. Brevotoxin binds to site 5, enhancing activation and preventing inactivation of the channel. The binding of delt channel inactivation (Catterall et al. 2007). Ample knowledge is also available for synthetic pyrethroid insecticides which bind to the sodium channel α-subunit altering the normal gating kinetics of VGSC. Initia specific binding site of pyrethroids were unsuccessful due to the extreme lipophilicity and the modest potency of pyrethroid radioligands. The subsequent developme demonstrated high affinity saturable binding to sodium channels in the brain. However, the high lipophilicity of pyrethroids still limited the sensitivity of the assay an single binding site responsible for pyrethroid action (Soderlund et al., 2002; Trainer et al., 1997). Despite these limitations, there remains overwhelming evidence tha alters sodium channel kinetics. Mutations in the VGSC in insects alter gating kinetics by decreasing the sensitivity of the channel to pyrethroids, and provide resistan 2000; 2001). In addition, the effects of pyrethroids are stereospecific where some isomers can interact with and modify channel function while other isomers are una channel kinetics (Soderlund 1985; Brown et al., 1988; Narahashi 1982). These properties of this class of insecticides on VGSCs are well established in the literature a by Soderlund et al. (2002).
KER2: KER2625 Altered kinetics of sodium channel leads to Disruption in action potential generation	Direct	The empirical evidence of KER2 is **STRONG**. As described in KE2, natural toxins like TTX bind to VGSC and block all electrical activity including action potentials. The potential generation has been widely demonstrated with a variety of other stressors (e.g., local anesthetics, anticonvulsants and other pharmacological agents (Hwa large body of literature on pyrethroids insecticides has confirmed their ability to alter action potential firing in both insect and mammalian peripheral and central in v These studies have been extensively reviewed (Soderlund et al., 2002; Narahashi et al., 1998; Bloomquist, 1996
KER3: KER3242 Disruption of action potential leads to altered neurotransmission during development.	Direct	The empirical evidence of KER3 is **STRONG**. There is abundant empirical evidence in the published literature supporting the basic biology underlying this KER. A vari insults can alter action potential generation and impair synaptic transmission (e.g. Seabrook et al., 1989; Joy et al., 1990; Staatz-Benson and Hosko, 1986; Hong et a and Dubocovich, 1988; Hossain et al., 2008; Shafer et al., 2008). These data have been generated in a wide variety of models from insects to mammalian models, in adult neuronal preparations. For a more detailed explanation, and examples of chemicals and mechanisms leading to altered neurotransmission, the readers are refe https://openbooks.lib.msu.edu/neuroscience/chapter/drug-and-toxin-effects/

KER4: KER3243 Altered neurotransmission during development leads to altered hippocampal anatomy	Direct	The evidence supporting this KER is considered **MODERATE.** Neurites of single cells in culture grow and retract dependending on the level of neuronal activation (Co Pharmacological block of action potentials by saxitoxin curtails synaptic transmission in PC12 and SH-SY5Y cell lines and inhibits neurite outgrowth (O'Neill et al., 20 activate synaptic transmission induces rapid input-specific changes in dendritic structure; however, these changes are reversed when neurotransmission is blocked (phenomena have been demonstrated in developing hippocampal cultures, dissociated neuronal cultures, organotypic slices and in intact organisms. The number, vo dendritic spines can all be altered with electrical stimulation. Spine growth is input specific, occurs only close to activated parts of the dendrite, and can be eliminate transmission at the postsynaptic receptor. Chronic blockade of neuronal activity leads to the reversible growth of dendritic spines in the hippocampus, while persiste spine structure contributes to the development and refinement of neural circuitry (Maletic-Savatic et al., 1999; Kirov and Harris, 1999). Cultured cortical neurons deprived of action potentials by an extended period of tetrodotoxin (TTX) treatment initially showed a marked increase in size and frequen the postsynaptic response to glutamate. Morphologically, these neurons retracted their dendrites, lost dendritic spines, and eventually degenerated over a period of morphological deterioration was prevented by blockade of glutamatergic AMPA receptors (Fishbein and Segal, 2007). As such, the block of action potential generatio neurotransmission impairment can lead to altered morphology by both direct and indirect means. Both higher and lower levels of activity can drive structural change in positive and negative directions, at ultrastructural and macrostructural scales. For example, ur elevated levels of electrical activity accompanying epilepsy reduce spine number (Geineisman et al., 1990). Sensory deprivation leading to lower activity levels in ne newly formed spines. Some examples include monocular deprivation in the mouse that eliminates electrical activity in visual cortex neurons in one hemisphere, doul spines in the binocular region of the same hemisphere (Hofer et al., 2009). Similarly, trimming the whiskers of rats to eliminate excitation of somatosensory neurons spines and an outgrowth of dendritic trees into the barrel field of the cortex (Vees et al., 1998). With a delay of several days, axons from the neighboring neurons, ur toward the deprived region. These adjacent neurons, although unaffected by the deprivation, experience altered activity levels, triggering their axonal growth. In bot models, structural plasticity is most pronounced during specific limited time windows in brain development. In the hippocampus, electrical stimulation of afferents al of pyramidal and granule cell neurons in vitro and in vivo (Kirov et al., 2004; Kirov and Harris, 1999; Geineisman et al., 1990; Maletoc-Savatic et al., 1999) and increa dentate gyrus (Chun et al., 2006; 2009). Activity-dependent structural changes in connectivity have been amply documented in adult networks and in the developing activity-dependent morphological growth and restructuring is paramount in development. Specific patterns of change may be different in the mature versus the deve activity is the trigger of structural change is not in doubt.
KER5: KER749 Hippocampal anatomy altered leads to hippocampal physiology altered	Direct	Empirical support for this KER is rated as **MODERATE**. There is no doubt that alteration of the structure of the hippocampus can lead to alterations of its function. Bo demonstrated that changes in glial and neuronal cell number or morphology impact physiological function in the hippocampus. Alterations in neurite number, length documented in hippocampal slice cultures with corresponding changes in synaptic function (Hosokawa et al., 1995). Chemical stressors (e.g., prenatal alcohol, devel nutritional deficits, and selective lesion models demonstrate a correlative link between altered structure and impaired synaptic function within the hippocampus (Gil- Hannigan, 2000; Palop et al., 2010; Ieraci and Herrera, 2007). Numerous examples of a direct linkage between hippocampal anatomy and hippocampal physiology a transgenic mouse models (e.g., Lessman et al., 2011), a few of which are detailed below. Mutations of the tyrosine kinase gene, Fyn, during development increased the number of neurons in the dentate gyrus and CA subfields of the hippocampus. Fyn mu impairments in long term potentiation in hippocampal CA1 whereas two other forms of short-term plasticity remained intact (Grant et al., 1992). Neuroregluin-2 (NRG2) is a growth factor that is highly expressed in the hippocampal dentate gyrus where it contributes to synaptogenesis of newborn granule cells. inducible microRNA targeting strategies have shown that suppression of NRG2 reduced synaptogenesis of inhibitory neurons and impaired dendritic outgrowth and n synapses. These anatomical alterations were accompanied by reductions in the amplitude of excitatory synaptic currents. The magnitude of the impairment was dep infection and could be eliminated with overexpression of NRG2 in this in vitro model (Lee et al., 2015). Brain-derived neurotrophic factor (BDNF) activation of CREB-activated gene expression plays a documented role in hippocampal synaptogenesis, dendrite formation, developing and adult nervous systems (Lessmann et al., 2011; Panja and Bramham, 2014). Jacob is a protein that translocates to the nucleus upon activation of BDN involved in both neuronal plasticity and neurodegeneration. Hippocampal neurons in culture derived from Jacob/Nsmf knockout mice exhibit shorter neurites with red synaptic contacts. This effect was specific to hippocampal neurons, as cortical cells derived from the same animals did not display these abnormalities. In vivo, these dendritic complexity of CA1 neurons, lower number of branches, and decreased spine density. Deficits in synaptic plasticity in the form of LTP accompanied these st al., 2016). Knockout of PSD-95 (a post-synaptic protein which regulates AMPA-R trafficking and synaptic maturation) impaired long term depression in CA1 neurons a Loss of PSD-95 thwarted the developmental increase in the number of functional AMPA-Rs expressing synapsis and prevented developmental changes in spine densi decreased spine size, a larger number of transient spines that were less stable), arresting synapses in a more immature state (Ehrlich et al., 2007). However, overex synaptic strength (by enhancing LTD (Schnell et al.,2002; Ehrlich & Malinow, 2004). IKK/NF-κB signaling is critically involved in synapse formation and spine maturation in the adult brain. IKK/NF- B blockade in hippocampus of mutant animals was ass mature spines and postsynaptic proteins (PSD95, SAP97, GluA1), and AMPAR-mediated basal synaptic transmission was suppressed. Exogenous Igf2 (IKK/NF-κB targ density and promote spine maturation (Schmeisser et al., 2012). In Alzheimer's Disease, amyloid-β protein accumulates in the hippocampus and leads to the formation of amyloid plaques, neuritic dystrophy and aberrant sprouting hippocampus. In a developmental germ-line knockout mouse model, high levels of amyloid-β induced aberrant neuronal network excitability and altered innervation in hippocampal plasticity were seen in the dentate gyrus without change in basal levels of synaptic transmission. In contrast, in area CA1, synaptic transmission was synaptic plasticity remained intact (Palop et al., 2007). Other evidence for a direct linkage between hippocampal anatomy and hippocampal physiology comes from the area of adult neurogenesis. The neurogenesis proce neurons on the hippocampus of the adult brain and is associated with enhanced hippocampal synaptic function and learning ability (Deng et al., 2010). Manipulation exercise and hormones can enhance neurogenesis and increase synaptic transmission and plasticity (Kapoor et al., 2015; Trivino-Paredes et al., 2016; Deng et al., 20 exists whereby increases in hippocampal neural activity serves to increase neurogenesis (Bruel-Jungerman et al., 2007, Bruel-Jungerman et al., 2009, Kameda et al., decrease hippocampal neurogenesis including exposure to antidepressants, hormone disruption, radiation, genetic ablation, stress, and alcohol are also associated w (Herrera et al., 2003; Saxe et al., 2006; Gilbert et al., 2016; Montero-Pedrazuela et al., 2006; Gil-Mohapel et al., 2010).
KER6: KER748 Hippocampal Physiology Altered leads to Cognitive Function Decreased	Direct	Empirical support for this KER is **STRONG**. The requisite of hippocampal integrity to optimal visuo-spatial context learning (i.e., episodic memory) in humans and spa documented. In vivo recording in conscious behaving animals has demonstrated activity-dependent neural changes taking place in the hippocampus during spatial le Garcia, 2007). Impairments in hippocampal function induced by drugs, chemicals, lesions, nutritional deficiencies, mutant or knock out models that cause changes in and hippocampal network activity, are coincident with deficits in spatial and context- based fear learning (O'Keefe and Nadel, 1978; Bannerman et al., 2014; Lynch, Similarly, treatments found to enhance or facilitate hippocampal synaptic transmission and plasticity are associated with improved learning and memory (Deng et al Andrade et al., 2015; Trivino-Paredes et al., 2016). A few examples of a large literature are briefly summarized below. It is well known that n-Methyl-d-aspartate (NMDA)-mediated glutamatergic synaptic transmission is essential for the induction of hippocampal synaptic plasticity in th form of plasticity by selective NMDA-receptors blockers impairs LTP and hippocampal tests of learning and memory (reviewed in Sweatt, 2016). Perturbation of hippo spatial learning have been reported in adult offspring following prenatal ethanol exposure (An and Zhang, 2015). Developmental morphine exposure caused decreas CA1 neurons fEPSPs that resulted in decreased maze performance (Aghighi et al., 2019). Developmental nutrition deficiency and hypoxic stress are both associated structure, altered EPSPs, and hippocampal based cognitive behaviors (Dumets et al., 2020; Zhuravin et al., 2019). Rodent models of developmental TH insufficiency in hippocampal synaptic transmission and plasticity and are coincident with deficits in learning tasks that require the hippocampus (Opazo et al., 2008; Gilbert and S al., 2016). There are also a number of mutant mouse models that have linked changes in hippocampal physiology with alteration in cognitive behaviors. The fyn mutant mouse displays impairments in hippocampal synaptic transmission and plasticity, as well as spatial learning deficits (Grant et al., 1992). Brain-derived neurotrophic factor (B synaptic plasticity deficits and learning impairments (Aarse et al., 2016; Panja and Bramham, 2014). In the Jacob/Nfsm model which also exhibits pronounced alterat hippocampal synaptic transmission and plasticity impairments were accompanied by deficits in contextual fear conditioning and novel location recognition tasks (Spi hydrocabon (AhR) knockout was shown to decrease hippocampal mossy fibers and also impair maze performance (Powers et al., 2005). Knockout of SALM4/Lrfn3, a synaptic adhesion molecule that modulates NMDA receptor function, increases NMDA-mediated currents and enhances contextual fear m level of performance could be restored via treatment with fluoxetine, a selective serotonin reuptake inhibitor (Li et al.,2021). Finally, a knockout of LIMK-1, a kinase a was shown to alter hippocampal spine morphology and LTP, with subsequent changes in fear behaviors and a spatial learning task (Meng et al., 2002). In humans, hippocampal physiology assessed using neuroimaging reveals activation of hippocampus upon engagement in spatial learning and episodic memory prov specific KEs (Burgess, 2002). In fMRI studies of congenitally hypothyroid children, or children born to women with altered thyroid function during pregnancy, changes during memory encoding and retention were observed and associated with memory impairments (Wheeler et al., 2012; 2015; Willoughby et al., 2013; 2014).

Quantitative Consideration

For the current AOP, quantitation of the relationships between KEs is limited. For KER 1 and KER 2, several software packages are available and give a clear and simple description of the voltage and current clamp methods. The software allows setting Na conductance levels and predicts resultant nerve action potentials. These models could be used to estimate the quantitative link between alterations in VGSC kinetics and action potential generation or disruption (MIE to KE2 in this AOP). In addition, as reported in this AOP, the quantitative relationship between the alteration of VGSC kinetics and the action potential generation has been modelled for tetramethrin but not for other pyrethroids. The timescale for the response-response of KER1 and KER2 should be considered as immediate.

Models for quantification of the remaining downstream part of the AOP are not currently available. For KER3, quantification could be feasible if future research uses the methods described in this AOP. By applying these methods, it would be possible to calculate the response-response relationship between the concentration-dependent perturbation of the action potential and the concentration-dependent downstream effect in the NNF assay. This investigation could be done at different stages of development.

A similar experimental approach could be taken to define the quantitative relationship between all remaining downstream KEs and the AO. However, different methodologies and metrics may be needed depending on the type of neuron, brain region and function in the central nervous system.

Considerations for Potential Applications of the AOP (optional)

The development of the new KE (KE 2005) referred to us 'Altered neurotransmission during development' has been a critical knowledge compilation in this AOP (see appendix C for detailed information on this KE and its KER). The regulatory relevance of the in vitro testable KE "Altered neurotransmission during development" is now further supported by the characterization of mechanistic KERs within this augmented AOP. These KERs link the KEs "altered hippocampal anatomy" and "altered hippocampal physiology" to the AO "decreased cognitive function". The biological plausibility and empirical evidence supporting these KERs have been assessed as moderate to strong.

This KE occurs in all life stages. As the balance between excitatory and inhibitory neurotransmission shapes hippocampal circuitry, any perturbation of this balance can lead to abnormal network activity (Cherubini et al., 2021). The methods and test systems used to measure abnormal network activity aresimilar in developmental or adult life stages. In addition, upstream KEs and pathways occurring in all life stages can lead to alteration in neurotransmission. However, in this AOP, KE 2005 pertains to the developmental period, because it is recognized that the biological and toxicological consequences can be different when disruption of neurotransmission occurs during development, versus adult life stages. It is well recognized that an infant's brain contains more neurons at birth than that of an adult, and the developing brain undergoes remarkable remodelling to achieve mature neural circuits via processes like apoptosis and synaptic pruning. As brain development matures further activity-dependent remodelling will strengthen circuits that prove more relevant and weaken others that are less frequently used. Such remodelling is more prominent in the first 2 years of life in humans and again during adolescence, with neural activity being a key driver for synaptic pruning. Thus, disruption of the formation of precise neural circuits during critical stages of brain development (i.e., perinatal and adolescence) may underlie neurodevelopmental disorders (Faust et al., 2021; see KER4 description and life stage applicability).

In mammals, it is well known that activity-dependent neuronal remodelling and the timing of this process depends on the brain region and cellular subtypes. This AOP focused on the hippocampal region (as detailed in Appendix C) since hippocampal circuits have been more extensively studied, particularly in relation to regulated chemicals. Furthermore, the hippocampus

has been causally linked to measurable AOs (e.g., learning and memory) in rodent models. While several model circuits for studying activity-dependent neuronal remodelling are available for many brain regions, future work is required to develop an AOP and KERs for other brain areas.

There are uncertainties in this new chemically agnostic AOP, including but not limited to knowledge gaps regarding quantitative relationships between KEs and the subsequent adverse impacts on cognitive functions, species extrapolation issues common to all animal based AOPs, and possible lower sensitivity of rodent cognition models commonly used in regulatory studies. This also holds true for developing AOPs for other brain regions, since this AOP is focused on the AO of altered hippocampal-based cognitive function. However, regarding the utility of the MEA-based neural network formation (NNF) assay (OECD, 2023) for use in chemical regulation, it is important to note that it uses cortical cell cultures. Thus, an effect on the NNF assay may not necessarily correlate with changes in the hippocampal-based spatial cognitive tests commonly used in regulatory in vivo DNT studies. It is biologically highly plausible that disturbed cortical cell based NNF generalizes to an adverse effect within other brain regions. For chemical regulation the derivation of relevant PoDs is more important than the prediction of any specific neurodevelopmental disorder at the organism level.

The newly developed KE 2005 can be measured using many methodologies that examine neural connectivity (i.e., neurotransmission), including the in vitro NNF assay. A standardized NNF test system to assess the potential impact of chemical exposure on neural network formation and function has been developed using rodent cortical neurons (Frank et al., 2017). This NNF assay is considered valid, biologically relevant and reliable by OECD (OECD, 2023) and the US EPA (US EPA, 2020a,b). An analysis for the regulatory use of the rodent primary cortical cell-based NNF assay and the additional 16 in vitro DNT assays has also been performed, and this may be contextualized with the uncertainties for in vivo data based uncertainties (Paparella et al., 2020).

The NNF assay represents a developing and relatively complex in vitro multi-cellular test system that includes many key neurodevelopmental processes, and provides a readout of neurophysiological function measured by changes in synaptic activity (i.e, general network activity, network bursting, network connectivity). If such activity is disturbed, it is likely caused by one or more upstream KEs (in this linear AOP or in a potential AOP network) that have previously been disturbed and not compensated at the (multi)cellular level. If these functional in vitro changes are large enough, they will disrupt neurological functions in an organism, ultimately eliciting negative effects. Within experimental systems, a positive response often holds greater regulatory relevance than a negative one, be it in vitro or in vivo. This is because none of these systems fully encompass all aspects of human higher cerebral functions, or the characteristics exhibited by humans in their natural state, including aspects such as metabolism, kinetics, molecular and cellular characteristics. Thus, positive effects observed in in vitro models, or in rodent in vivo studies, should be considered indicators of toxicity. Their impact in real life human conditions depends on additional factors such as (epi)genetic background, socioeconomic status, diet, lifestyle, stress, infections and chemical co-exposures.

The direct regulatory relevance of these disruptions, if integrated with other toxicological information, can be used to derive a PoD, which will be the basis for setting a health-based guidance value. To facilitate regulatory use in decision making, an agreed tiered testing strategy approach for use of in vitro data would be helpful. This approach should include the MEA-based NFF assay as well as the remaining assays in the DNT IVB together with interpretive guidance on the MEA/NFF outcomes for quantitative human health risk assessment.

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Appendix 1

List of MIEs in this AOP

Event: 1353: Binding to voltage-gated sodium channel

Short Name: Binding to VGSC

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:215 - Molecular events lead to epilepsy	KeyEvent
Aop:230 - presynaptic neuron 1 activation to epilepsy	KeyEvent

AOP ID and Name	Event Type
Aop:442 - Binding to VGSC during development leads to cognitive function decrease	MolecularInitiatingEvent
Aop:489 - Inhibition of voltage-gated sodium channels leading to decreased cognition	MolecularInitiatingEvent

Stressors

Name
Pyrethrins and Pyrethroids

Biological Context

Level of Biological Organization
Molecular

Cell term

Cell term
eukaryotic cell

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Vertebrates	Vertebrates		NCBI
Invertebrates	Invertebrates		NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Male	
Female	

VGSCs are present in many different cell types of the central nervous system (CNS), including neurons, oligodendrocytes, Schwann cells (Baker, 2002; Jessen and Mirsky, 2005; Ritche, 1992; Chiu, 1991) and microglia (Jung et al., 2013; Black and Waxman reviewed in Hossain et al., 2017; Paez et al., 2009; Berret et al., 2017).

Moreover, every cell within living organisms actively maintains a low intracellular sodium concentration that is 10–12 times lower than the extracellular concentration. The cells then utilize this transmembrane sodium concentration gradient as a driving force to produce electrical signals, and if the driving force is sufficiently strong, an AP is produced. The protein family comprising VGSC (Navs) is essential for such signaling and enables cells to change their electrical status in a regenerative manner and to rapidly communicate with one another. The existence of VGSC was first predicted from studies of electrical activity in squid giant axon and later identified through molecular studies in the electric eel. Since then, these proteins have been observed in organisms ranging from bacteria to humans (Chaihne, 2018).

Sodium channels consist of highly processed α subunit, which is approximately 260 kDa, associated with auxiliary β subunits of 33–39 kDa. Sodium channels in the adult CNS and heart contain a mixture of β1–β4 subunits, while sodium channels in adult skeletal muscle have only the β1 subunit. Nine different VGSC have been identified using electrophysiological recording, biochemical purification, and cloning (Catterall, 2007; Catterall, 2012).

Nomenclature of the different sodium channel alpha (pore-forming) subunits is based on a numerical system to define subfamilies and subtypes based on similarities between the amino acid sequences of the channels. In this nomenclature system, the name of an individual channel consists of the chemical symbol of the principal permeating ion (Na) with the principal physiological regulator (voltage) indicated as a subscript (Nav). The number following the subscript indicates the gene subfamily (currently only Nav 1), and the number following the full point identifies the specific channel isoform (e.g. Nav 1.1). This last number has been assigned according to the approximate order in which each gene was identified. Splice variants of each family member are identified by lower-case letters following the numbers (e.g. Nav 1.1a). (Catterall, 2012).

In mammals, numerous neuronal VGSC are expressed in the adult and developing brain. Evidence from mutation and knockout animal models demonstrates that perturbation of VGSC function during development impairs nervous system structure and function, disrupts muscle function, pain reception, and cardiac rhythm (Chahine, 2018). VGSCs show complex regional and temporal ontogeny in mammals. Table 1, from Shafer et al., 2005 provides an overview about the alpha subunits and their developmental and tissue expression pattern. Pyrethroid interactions with Nav1.1 (James et al., 2017), Nav1.3 (Meacham et al., 2008; Tan and Soderlund 2009), Nav1.6 (Tan and Soderlund, 2010), Nav1.7 (Tan and Soderlund, 2011) and Nav1.9 (Nutter and Cooper, 2014; Bothe et al., 2021) channels.

Table 1. Sodium channel α subunit nomenclature and effects of pyrethroids.[a]

α subunit	Older names	TTX sensitivity	Tissue expression	Developmental expression	Effect of pyrethroids
$Na_v1.1$	Rat I, HBSCI, GPBI, SCN1A	TTX-S	CNS, PNS, Purkinje, HP pyramidal cells, spinal motor neurons, somatic localization	Not detected in HP during development, detectable in CB Purkinje cells at PND15, detected at PND2 in SC; strong expression in motor neurons[b]	Not tested to date
$Na_v1.2$	Rat II, HBSCII, HBA	TTX-S	CNS, forebrain, substantia nigra, HP mossy fibers, CB molecular layer, axonal localization	In HP, increase between GD17 and PND30; in CB granule cells on PND15 and Purkinje cells on PND2; detected at all ages in SC[b] Splice variant expressed during development[c]	Cypermethrin-induced tail currents detectable at > 30 nM in rat 1.2 (adult splice variant) co-expressed with β_1 subunits; reported insensitive to permethrin or cismethrin[d]
$Na_v1.3$	Rat III	TTX-S	CNS and DRG	HP expression at GD17, increasing at PND2, then decreasing to barely detectable at PND30. Detected at GD17 in CB neuroepithelium, decreasing thereafter, similar in SC[b]; developmentally regulated splice variant[e]	Not tested to date
$Na_v1.4$	SkM1, μ1	TTX-S	Skeletal muscle	Increases with age[f]	Only slightly modified by 10 μM deltamethrin when expressed in HEK 293t cells[g]
$Na_v1.5$	SkM2, H1	TTX-R	Uninnervated skeletal muscle, heart, brain	mRNA expressed in rat PND0 limbic structures and medulla; expressed in fetal and adult human brain[h]	Not tested to date
$Na_v1.6$	NaCh6, PN4, Scn8a, CerIII	TTX-S	CNS, DRG (all diameter neurons), node of Ranvier–peripheral nerve	Truncated form expressed from GD12 to PND7, full-length mRNA expression is slight at GD14 and increases with age[i]	Not tested to date
$Na_v1.7$	NaS, hNE-NA, PN1	TTX-S	DRG (all diameter neurons) CNS, Schwann cells	All DRG neurons at PND2, increased during development[b]	Not tested to date
$Na_v1.8$	SNS, PN3, NaNG	TTX-R	DRG (small diameter neurons)	Expression beginning at GD15 with adult levels by PND7; largely in unmyelinated C-fibers[j]	Sensitive to both cismethrin and cypermethrin at thresholds of 500 nM and 30 nM, respectively[k]
$Na_v1.9$	NaN, SNS2, PN5, NaT, SCN12A	TTX-R	DRG (small diameter neurons)	Expression beginning at GD17 with adult levels by PND7; largely in unmyelinated C-fibers[j]	Not tested to date
Na_x	$Na_x2.1$, $Na_x2.3$ Na-G, SCL11	?	Heart, uterus, skeletal muscle, astrocytes, DRG	Transient between PND2 and 15 in HP; peak expression at PND2 in CB, SC; large DRG neurons, GD17 to PND30[b]	Not tested to date

Abbreviations: CB, cerebellum; CNS, central nervous system; DRG, dorsal root ganglion; GD, gestation day; HP, hippocampus; PND, postnatal day; PNS, peripheral nervous system; SC, spinal cord; TTX, tetrodotoxin; TTX-R, TTX resistant; TTX-S, sensitive to TTX.
[a]Data in the first four columns are based on information presented by Goldin et al. (2000) and Novakovic et al. (2001). [b]Felts et al. (1997). [c]Sarao et al. (1991). [d]Smith and Soderlund (1998). [e]Gustafson et al. (1993). [f]Kallen et al. (1990). [g]Wang et al. (2001). [h]Donahue et al. (2000). [i]Plummer et al. (1997). [j]Benn et al. (2001). [k]Smith and Soderlund (2001).

ß1b and ß3 expression is high during prenatal and early postnatal period in nervous system mammals, followed by increased expression of ß1, ß2 and ß4 in the first postnatal week which then persists through adulthood. While different cell types in the brain express different ß subunits, the ß1 subunit is ubiquitously expressed with moderate heterogeneity. Its subcellular localization provides specific functionalities, e.g. high density of ß1 at the nodes of Ranvier modulates surface expression and gating of the VGSCα subunit while in the paranodal region ß1 mediates axonal-glial cell adhesion. The ß2 protein shares some similar expression pattern with ß1 and appears to provide responsiveness to inflammatory and neuropathic pain in the

peripheral nervous system (PNS). In contrast ß3 mRNA and protein are expressed ubiquitously thought the developing CNS and in adult mice it is greatly reduced except for some structures like the hippocampus. This differs in human brain, where ß3 remains highly expressed throughout adulthood. The expression profile of ß4 is mostly restricted among the ß subunits, and often related to neurons with spontaneous or burst firing APs. Finally, β subunits are also expressed in various glia where they may function as cell adhesion guides and cues for neurodevelopment, including coordinating neurite outgrowth, axonal fasciculation, and neuronal migration (Hull et Isom 2018). Importantly, co-expression of β subunits with the α subunit modulates the function of the α subunit and can influence the binding of various ligands to the α subunit (Tan et al., 2011). In general, embryonically expressed forms of VGSCs are replaced by expression of adult forms as neurodevelopment proceeds.

Due to this complex ontogeny of VGSCs it is currently not possible to specify which VGSCs subtypes and which developmental stages are particularly essential and thus important for this AOP.

Key Event Description

Due to their critical role in neuronal function, sodium channels are known molecular targets of neurotoxins and neurotoxicants (Wakeling et al., 2012). The essentiality of sodium channels in nerve conduction comes from classic literature on tetrodotoxin (TTX). TTX is a sodium channel blocker that inhibits the firing of action potentials in neurons by binding to the voltage-gated sodium channels (VGSC/NaV) in nerve cell membranes. This action blocks the passage of sodium ions into the neuron, ions responsible for the rising phase of an action potential (AP). There is strong evidence implicating a similar TTX-like of pyrethroid insecticides on VGSC. This block of VGSC is supported by an extensive body of literature on the action of pyrethroid insecticides on mammalian sodium channels. Binding studies using radioactive pyrethroid demonstrated specific binding of the pyrethroid to rat brain VGSC α subunits (Trainer et al., 1997).

Ion channels are integral membrane proteins that are critical for neuronal function. They form pores in the plasma membrane that allow certain ions to travel across the membrane along their electrochemical gradient. Ion channels that open in response to a change in membrane voltage potential are called ‘voltage-gated’ ion channels. Channels that open in response to binding using a chemical signal or molecule are ‘ligand-gated’ ion channels. In neurons, ion channels of both types are essential for chemical communication between cells, i.e., synaptic transmission. Ion channels also function to maintain membrane potential and initiate AP to propagate electrical impulses. VGSC are therefore responsible for AP initiation and propagation in most excitable cells, including nerve, muscle and neuroendocrine cell types. It is important to note that functional VGSC are present in both grey and white matter in the brain. Approximately 50% of white matter oligodendrocyte precursor cells receive synaptic inputs and can produce trains of VGSC-dependent APs (Fields, 2008). VGSC are also present on microglia where they contribute to the release of major pro-inflammatory cytokines (Hossain et al., 2017).

Mammalian VGSC are composed of one α and two β subunits. Ten separate α subunits (Ogata and Ohishi, 2002) and four different β subunits (Isom, 2002) have been identified and are expressed in tissue-, region- and time- specific manners. The diverse functional roles of VGSCs depend on the numerous potential combinations of α and β subunits (Ogata and Ohishi, 2002). The type of VGSCs expressed in different cell types and regions, their sensitivity and their functional role, all contribute to the manifestation of toxicity and age-dependent sensitivity, of chemicals acting at this site.

How it is Measured or Detected

Interaction of compounds with VGSC can be measured directly with radioligand binding (Trainer et al 1997), while the expression and localization of VGSC on different cell types can be assessed using immunohistochemical methods. The following discussion focuses on interactions between VGSC and pyrethroids, but similar data exist for other compounds that bind to VGSC. Several other approaches provide indirect evidence of interactions of chemicals with VGSC. The published literature contains hundreds of reports identifying point mutations in VGSC that alter both the effects on the channel as well as the sensitivity to pyrethroid toxicity. Both increased and decreased modification of the insect and mammalian VGSC by pyrethroids have been demonstrated, specific action dependent on the location and type of point mutations (e.g. Vais et al., 2000; 2001). Finally, the demonstration of stereo-specific effects of the pyrethroids on binding (Soderlund 1985; Brown et al., 1988) as well as electrophysiological responses (Narahashi 1982; Narahashi1996; Narahashi., 200; Narahashi., 2002) also supports interaction of VGSC and pyrethroids. A model for binding of pyrethroids in insect VGSC has been developed (O’Reilly et al., 2006). Together, these observations provide strong evidence of pyrethroid binding to VGSC (for additional review, see Field et al 2017).

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List of Key Events in the AOP

Event: 1977: Disruption of sodium channel gating kinetics

Short Name: Altered kinetics of sodium channel

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:442 - Binding to VGSC during development leads to cognitive function decrease	KeyEvent
Aop:489 - Inhibition of voltage-gated sodium channels leading to decreased cognition	KeyEvent

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
eukaryotic cell

Organ term

Organ term
nervous system

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Vertebrates	Vertebrates		NCBI
Invertebrates	Invertebrates		NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Male	
Female	

Ion channels are essential for the initiation and propagation of APs in excitable cells in both vertebrate and invertebrate species. In neurons, ion channels are essential for chemical communication between cells, or synaptic transmission. Ion channels also function to maintain membrane potential and initiate and propagate electrical impulses. VGSC are a target of natural and synthetic chemicals and disruption of the gate kinetics has been characterized in insects and mammalian cells (Soderlund et al., 2002).

Key Event Description

Action potentials (AP) are a temporary shift (from negative to positive) in the neuron's membrane potential caused by ions flowing in and out of the neuron. During the resting state, before an action potential occurs, voltage-gated sodium and potassium channels are predominantly closed. These gated channels only open once when an action potential has been triggered. They are called 'voltage-gated' because they are open and close depending on the voltage difference across the cell membrane. VGSCs have two gates (gate m and gate h), while the potassium channel only has one (gate n). Gate m (the activation gate) is normally closed and opens when the cell membrane potential starts to get more positive (depolarizes). Gate h (the deactivation gate) is normally open, and swings shut when the cell membrane potential gets too positive. Gate n is normally closed, but slowly opens when the cell is depolarised (very positive). VGSCs exist in one of three states: Deactivated (closed), activated (open) and inactivated (closed) – at rest, channels are (Figure 1) .

Modifications of the sodium channel gating have been studied using voltage and patch clamp experiments in different models (Ruigt et al., 1987). Prolongation of the sodium current is mainly due to the reduced rate of closure of a fraction of the sodium channel population and is characterized by a 'tail curent'. In neuroblastoma cell preparations, chemical stressors including deltamethrin and other type II pyrethroids, induce a slow tail current with a relatively long time constant. The rate at which sodium channels close during the pyrethroid-induced slow tail current depends not only on pyrethroid structure, but also on the duration of exposure, temperature and membrane potential (Ruigt et al., 1987; Narahashi., 2002; Soderlund., 2002).

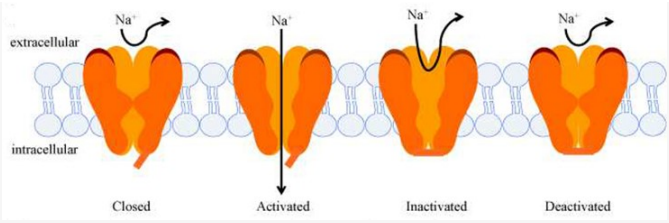

Figure 1. The three existing states of the VGSCs: Deactivated (closed), activated (open) and inactivated (closed).

How it is Measured or Detected

Typically, VGSC function is measured using electrophysiological approaches, as only these have sufficient temporal resolution to evaluate channel function. Voltage-clamp techniques typically use two microelectrodes, allowing control of the membrane potential ('clamping') and recording of transmembrane currents that result from ion channel opening and closing (Guan et al., 2013). Pharmacological approaches and modifications of the ionic composition of the solution are used to isolate currents passing through VGSC from other types of current in the neuron.

In the patch-clamp technique, a highly sensitive version of the voltage-clamp technique, a single glass microelectrode is attached to a neuron to form a tight seal between the glass pipette tip and the cell membrane. In this case, a single electrode controls voltage and passes current (Molleman, 2003). Typically, the current measured is the sum of currents flowing through the entire population of channels in this patch of membrane, the 'whole cell' patch configuration (Hamill et al., 1981). Some configurations of patch clamp technique can measure current flowing through a single ion channel. Most studies utilizing this technique involve in vitro or ex vivo measurements.

Other approaches can be used to indirectly measure VGSC function, including radiotracer flux, fluorescent approaches, and calcium imaging. While these approaches can provide useful information in many cases, they are not direct nor do they have sufficient resolution to fully describe VGSC function.

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Event: 1983: Disruption, action potential

Short Name: Disruption in action potential generation

Key Event Component

Process	Object	Action
action potential		disrupted

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:442 - Binding to VGSC during development leads to cognitive function decrease	KeyEvent

Biological Context

Level of Biological Organization
Cellular

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Vertebrates	Vertebrates	High	NCBI
Invertebrates	Invertebrates	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Male	
Female	

Action potentials or nerve impulses are rapid and transient electrical activity that are propagated in the membrane of excitable such as neurons and muscle cells. The same principal mechanism exists in all cells and therefore is independent of sex. Action potentials are present from fetal stages on in vertebrates and they are also present in invertebrates.

Key Event Description

Generation of action potentials (APs)

The action potential is a transient depolarization and repolarization of the membrane that occurs in electrically excitable cells (neurons, cardiac cells, muscle). Due to unequal distribution of charged ions between the inside and outside of the cell, a voltage difference (potential) exists between the intracellular and extracellular sides of the cell membrane. At "resting" levels of activity, this membrane potential is about -70 millivolts. Typically, sodium ions are much higher outside of the cell while potassium ions are higher inside of the cell. The action potential is initiated by a depolarizing stimulus that results in the opening of voltage-gated sodium channels in the membrane. Once a few sodium channels open, this triggers local depolarization opening other nearby VGSC and induction of a rapid depolarization to positive potentials (e.g. +20 mV) of the membrane. This rapid depolarization is due to influx of positively charged sodium ions into the cell through the VGSC. Sodium channels rapidly "inactivate", closing even if the membrane remains depolarized, limiting the amount of sodium entering the cell and stopping additional depolarization. The VGSC induced depolarization of the membrane causes voltage-gated potassium channels to open, which results in positively charged potassium ions exiting the cell to repolarize the membrane to its resting potential. In myelinated neurons, myelin introduces insulation around the axon that allows depolarization to spread further down the axon with great efficiency. Sodium channel expression is higher around specialized "nodes" in the myelin sheath, nodes of Ranvier. The high concentration of VGSCs at these nodes increase

the probability that a sufficient number of VGSC will open on depolarization to reach the threshold for firing an action potential. In this manner the sequestering of VGSC at the node allows the electrical impulse to quickly jump from node to node along the length of the axon, increasing the speed of propagation of the action potential.

The process described above is highly conserved across electrically excitable cells and is described in a generic manner here. Due to the diversity of different neuron types and expression of different combinations of ion channels across those neuron types, differences in the shape and temporal patterns of APs are observed across different cells in the nervous system. The basic components of the action potential as described above are well conserved in neurons from invertebrates to vertebrates including mammals and humans.

For an easy-to-read summary description including figures of action potential generation and propagation, see e.g.

- https://openbooks.lib.msu.edu/neuroscience/chapter/action-potentials/
- https://pressbooks.umn.edu/sensationandperception/chapter/action-potentials/

How it is Measured or Detected

The action potential is a cycle of membrane depolarization, hyperpolarization and return to the resting value. It is measured most directly using electrophysiological approaches, which allow measurements to be made on a time scale that is consistent with the speed at which these events occur (milliseconds). Other approaches allow for more indirect assessments of APs using optical and other measures. Typically, these optical approaches do not have the temporal resolution of electrophysiological measurements.

Electrophysiological Techniques For Measurements of Action Potentials

There are a wide variety of electrophysiological techniques that allow for action potential measurement. At their core, all of them allow the recording of changes in either membrane potential or currents flowing across the membrane, and all are capable of doing so with high temporal resolution (milliseconds) necessary to record APs. Different configurations each have inherent advantages and disadvantages and the selection of the appropriate technique depends on the specific questions to be addressed by an experiment. All these approaches make use of one or more electrodes to measure the electrical responses (changes in membrane voltage or current) in a cell or group of cells. The electrodes can be of various sizes and shapes, and may be placed inside the cell (intracellular recordings), on the cell (patch clamp recordings), or adjacent to the cell (extracellular recordings).

In patch recording, in contrast to evaluating specific channel activity as described in KE1 for VGSC activation, to evaluate AP, the current clamp configuration is commonly employed. Also distinct from KE1, pharmacological manipulations are not applied so that all channel types can contribute to the AP response - AP requires both sodium and potassium ion flow. The action potential is reflected in a rapid fluctuation in voltage.

In the intracellular recording, sharp glass microelectrodes are inserted directly into the intracellular space of the neuron and membrane voltage is measured. Membrane voltage increases dramatically once a threshold depolarization is reached and an action potential is reflected in a short duration steep increase in voltage followed by a rapid fall. Compared to patch clamp, sharp electrode intracellular recording is more difficult to perform, but allows recordings to be obtained for much longer periods of time. They measure the synaptic signals of cells with a high signal-to-noise ratio.

Rather than on or in the cell, extracellular recordings show changes in the activity of several cells surrounding a microelectrode. Alterations to the position and size of this electrode will change the nature of the measurement. These are referred to as unit recordings where APs are characterized by high frequency of activity on msec timescale. Often, action potential signals from multiple cells are recorded on the same electrode and one cell distinguished from the other using a process called spike sorting. Spike sorting uses computer algorithms to analyze the waveforms of the electrical activity and separate them based on their temporal profile, amplitude and other characteristics.

Microelectrode arrays (MEAs) are a form of extracellular recording, consisting of chips that contain multiple electrodes, typically arranged in a small grid. Rather than recording from a single electrode, action potential signals can be recorded from multiple electrodes simultaneously. The number of electrodes ranges from tens to thousands depending on the spatial resolution of the array and type of data required by the experiment. Different types of arrays can be used for a wide variety of in vitro and in vivo applications. MEA recordings provide multiple parameters of electrical activity, with firing rate and bursting rates as the most common to characterize APs.

Local field potentials are also extracellular recordings, but measure the synchronized electrical potential of a group of cells whose source may be difficult to determine. The signals from these cells will overlap and the recording will be a sum of all of the electrical activity. Commonly used in laminated structures with known anatomical inputs, stimulating electrodes are placed on the input presynaptic axonal field and electrical responses induced after a short synaptic delay represent neurotransmission from pre to postsynaptic neurons.

Optical measurements of action potential

A variety of optical techniques are used as indirect measurement of the action potential. These have the advantage of being higher throughput than electrophysiological approaches, but the disadvantage of having a slower temporal resolution. They are highly correlated with action potential generation, but are subject to some confounders and do not possess the temporal resolution of electrophysiological approaches. These include the use of Na+ or Ca++ sensitive dyes that fluoresce in response to the binding of one of these ions. Single APs are not detected, but changes in bulk ion concentration over a finite period of time primarily reflect the firing rate of the cells. Another optical technique uses dyes that are sensitive to changes in voltage or fluorescence resonance energy transfer (FRET) using specialized fluorophores that respond based on changes in membrane potential. Large changes in FRET fluorescence are indicative of changes in electrical activity. Under the correct conditions, FRET fluorescence can reflect changes in action potential generation.

For additional information on these techniques see Khadria, 2022 and Ogden, 1994.

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Event: 2005: Altered neurotransmission in development

Short Name: neurotrasmission in development

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:442 - Binding to VGSC during development leads to cognitive function decrease	KeyEvent

Biological Context

Level of Biological Organization

Cellular

Cell term

Cell term

neuron

Organ term

Organ term

brain

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Male	
Female	

The process of neurotransmission is generally similar in structure/function across most taxa with nervous systems (Libenskind et al. 2017; Roschchina 2010). This includes studies using both *in vitro* and *in vivo* methods. In vertebrates, synaptic transmission usually travels in one direction. The process of synaptic transmission has been well-studied and is described in many standard neurophysiology textbooks.

Components of the synapse include (a) a presynaptic module, in which calcium signals are transduced into chemical secretions (known as excitation–secretion coupling); (b) a postsynaptic module (postsynaptic density), which comprises the proteins that support the specialized postsynaptic membrane and the signalling that goes on there; and (c) a module that determines the specific wiring diagram of neurons during development (axonogenesis). For the module "c", during development, and after injury, axons must grow and find their correct synaptic targets. Electrical activity in neurons and neural networks can stabilize structural connections at the synapse, while loss of activity leads to elimination of synapses. The proteins responsible for this targeting include secreted and membrane-bound signals and receptors that have not been as well studied in an evolutionary framework as for the other modules.

Despite its apparent specialization for neuronal signalling, the excitation–secretion system in neurons comprises many ancient gene families. However, like the transduction module, these gene families are often used differently in the various animal lineages. The proteins involved in docking and in recycling are, for the most part, conserved across eukaryotes (Liebenskind et al. 2017).

Several neurotransmitters have been found not only in animals, but also in plants and microorganisms. Thus, the presence of neurotransmitter compounds has been shown in organisms lacking a nervous system and even in unicellular organisms. Today, we have evidence that neurotransmitters, which participate in synaptic neurotransmission, are multifunctional substances participating in developmental processes of microorganisms, plants, and animals (Roschchina 2010). In the brain, many neurotransmitters also act as trophic factors in early brain development.

The neurotransmission wiring code, including excitation–secretion coupling, postsynaptic density and axonogenesis is present across multiple taxa and represents a fundamental brain developmental process.

Key Event Description

The arrival of the nerve impulse at the presynaptic terminal of the nerve's axon stimulates the release of neurotransmitter into the synaptic cleft. The neuron is a secretory cell and the secretory product, the neurotransmitter, is released to span the distance between neurons, the chemical synapse.

Neurotransmitters synthesized by the neuron are stored in the presynaptic element, inside the pre-synaptic vesicles. Release of neurotransmitter can be described by probabilistic principles. The probability of neurotransmitter release is very low under normal "resting" conditions but increases dramatically upon depolarization of the axonal nerve terminal by an action potential (AP). AP depolarization of the presynaptic site of the axon terminal causes voltage gated Ca2+ channels to open. Calcium ions entering the cell initiate a signalling cascade that causes small membrane-bound vesicles, called pre-synaptic vesicles, containing neurotransmitter molecules to fuse with the presynaptic membrane. Neurotransmitters are then released, diffuse across the space between the presynaptic and postsynaptic neurons, the synaptic cleft, and bind to their appropriate receptors on the postsynaptic membrane. Signalling is then terminated by three different mechanisms: diffusion of the neurotransmitter out of the synaptic cleft, degradation of neurotransmitter by specific enzymes (e.g. acetylcholinesterase), or reuptake of the neurotransmitter by glia cells and the presynaptic neuron.

In principle different neurons excrete excitatory or inhibitory neurotransmitters, inducing in the postsynaptic membrane either a depolarisation or hyperpolarisation, respectively. In consequence these actions either trigger or impede the generation of a new postsynaptic depolarization. Neurons integrate the various excitatory and inhibitory signals they receive from the large number of synapses with their presynaptic network, resulting in a net signalling event in their postsynaptic targets.

Disruption of neurotransmission during development can result in permanent changes in nervous system function.

How it is Measured or Detected

Neurotransmission can be measured by a wide variety of different approaches. The same technologies described in KE2 for AP generation can be used to measure neurotransmission by applying different protocols. These include patch clamp, intracellular and extracellular recordings, microelectrode array (MEA) recordings. Depending on the type of recording used, electrophysiological techniques capture presynaptic events such as the action potential arriving at the terminal that induces a release of neurotransmitter, or post-synaptic events, such as post-synaptic excitatory or inhibitory responses. These responses are induced by binding of neurotransmitter to postsynaptic membrane receptors. These electrical signals are the consequence of neurotransmitter diffusion from the pre- to the postsynaptic element, binding of neurotransmitter molecules to postsynaptic receptors and induction of the postsynaptic current.

Biochemical assessment of neurotransmission (i.e. in vivo microdialysis or in vitro measurement of neurotransmitters released into the media) are also common and well described in the literature.

Patch Clamp

Intracellular recordings measure neurotransmission following stimulation of presynaptic neurons via excitatory and inhibitory currents by electrodes positioned inside the postsynaptic neuron. Similar currents can be assessed using patch clamp techniques.

In extracellular field potential recordings stimulating electrodes placed on the input presynaptic axonal field induce after a short synaptic delay a response in the postsynaptic cells reflecting neurotransmission.

In microelectrode arrays, neurotransmission is reflected by parameters including synchronized network activity, correlation of neuronal activity across multiple electrodes, and activity evoked by direct stimulation.

As described above for KE2, Action Potential Generation, optical approaches can also be used to measure neurotransmission, such as the use of pH sensitive dyes that are incorporated into the pre-synaptic vesicles, dyes that change fluorescent properties once released into the synaptic cleft due to a difference in pH between the vesicle compartment and the synaptic cleft.

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Event: 757: Hippocampal anatomy, Altered

Short Name: Hippocampal anatomy, Altered

Key Event Component

Process	Object	Action
brain development	hippocampal formation	morphological change

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:42 - Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals	KeyEvent
Aop:8 - Upregulation of Thyroid Hormone Catabolism via Activation of Hepatic Nuclear Receptors, and Subsequent Adverse Neurodevelopmental Outcomes in Mammals	KeyEvent
Aop:134 - Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals	KeyEvent
Aop:152 - Interference with thyroid serum binding protein transthyretin and subsequent adverse human neurodevelopmental toxicity	KeyEvent
Aop:300 - Thyroid Receptor Antagonism and Subsequent Adverse Neurodevelopmental Outcomes in Mammals	KeyEvent
Aop:442 - Binding to VGSC during development leads to cognitive function decrease	KeyEvent

Stressors

Name
Propylthiouracil
Methimazole

Biological Context

Level of Biological Organization

Tissue

Organ term

Organ term

brain

Domain of Applicability

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
During brain development	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

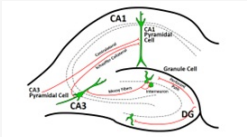
The hippocampus is generally similar in structure function across most mammalian species (West, 1990). The vast majority of information on the structure of the hippocampus is from mice, rats and primates including humans.

Key Event Description

The hippocampus is a major brain region located in the medial temporal lobe in humans and other mammals (West, 1990). Developmentally it is derived from neuronal and glial cells in the neural tube and differentiates in the proencephalon and telencephalon. The hippocampus is a cortical structure, but only contains 3-layers, distinct from the 6-layered neocortical structures. For this reason, it is known as archicortex or paleocortex meaning old cortex. In humans, at the macro level, the structure is identified as early as fetal week 13 and continues to mature until 2 to 3 years of age (Kier et al., 1997), with continuing slow growth thereafter until adult ages (Utsunomiya et al., 1999). In rodents, the hippocampus begins to form in mid-gestation, with the CA fields forming in advance of the dentate gyrus (Altman and Bayer, 1990a; 1990b). Generally speaking, the primary structural and functional development of the hippocampus occurs in the third trimester of pregnancy in humans, whereas in rodents, much of the maturation of the CA fields and almost all dentate gyrus occurs in the first 2-3 postnatal weeks.

The structure of the hippocampus has been divided into regions that include CA1 through CA4 and the dentate gyrus. The principal cell bodies of the CA field are pyramidal neurons, those of the dentate gyrus are granule cells.

The major input pathway to the hippocampus is from the layer 2 neurons of the entorhinal cortex to the dentate gyrus via the perforant path forming the first connection of the trisynaptic loop of the hippocampal circuit (Figure 2). Direct afferents from the dentate gyrus (mossy fibers) then synapse on CA3 pyramidal cells which in turn send their axons (Schaeffer Collaterals) to CA1 neurons to complete the trisynaptic circuit. Information from the CA fields then passes through the subiculum entering the fiber pathways of the alveus, fimbria, and fornix and is routed to other areas of the brain (Amaral and Lavenex, 2006). Through the interconnectivity within the hippocampus and its connections to amygdala, septum and cortex, the hippocampus plays a pivotal role in several learning and memory processes, including spatial behaviors. The primary input pathway to the CA regions of the hippocampus is from the septum by way of the fornix and direct input from the amygdala. Reciprocal outputs from the hippocampus back to these regions and beyond also exist.



Trisynaptic circuit of hippocampal formation. The dentate gyrus (DG) receives input from entorhinal cortex neurons whose axon travel as a fibre tract called the performant path. These axons terminate on granule cell dendrites of the DG. The 2nd synapse of the circuit is mossy fibre axons of DG granule cells that synapse on pyramidal cells of the CA3 subfield of the hippocampus. The 3rd synapse of the circuit is the axons of CA3 neurons, the Schaffer Collateral that synapse on dendrites of CA1 pyramidal cells. Axons from the contralateral CA3 field cross the midline to also innervate the CA1 neurons.

Figure 2.

At the cellular level, the components of the mammalian hippocampus undergo typical stages of neurodevelopment. With each developmental time window, distinct patterns of gene transcription and protein expression appear, corresponding to cell proliferation, differentiation, migration, synapse formation, and terminal neuronal/glial maturation, culminating in the structural formation of a neuronal network (Mody et al., 2001; Laeremans et al., 2013). The principal neurons of the CA fields develop in advance of the principal cells of the dentate gyrus and the genes and proteins controlling the distinct phases are expressed at different stages in these two sub-regions (Altman and Bayer, 1990 a, b; Laeremans et al., 2013). In the rodent brain, almost all neurons show extensive growth and differentiation on axons and dendrites during the first postnatal week. These cellular changes are marked by rapid protein expression specific for different neuronal and glial subtypes including cytoskeletal proteins (e.g. cofilin, actins, tubulins etc), production of cell adhesion molecules, and extracellular matrix formation which are critical structural elements of a neuronal network.

As neurons mature, they extend dendritic processes that lengthen and branch, the ends of which broaden to form a spine head. Dendritic spines form the postsynaptic structural component of most excitatory synapses in the mammalian brain, including hippocampus. The spine head has a greater potential for connectivity and synapse formation (Dailey and Smith, 1996; Fiala et al., 1998; Hardy, LR and Redmond, 2008, Pfeiffer et al., 2018). The postsynaptic density-95 (PSD-95) is one of the key proteins involved in dendritic spine maturation, clustering of synaptic signalling proteins, and ultimately mediating synaptic transmission. It also plays a critical role in regulating dendrite outgrowth and branching and formation dendritic spines.

As the hippocampus matures during the postnatal period hippocampal circuits become more active and exhibit increased activity-dependent plasticity. Many genes and proteins are upregulated during this phase of development, especially molecules involved in the axon guidance (e.g. BDNF/CREB) (Hinkemeyer et al., 2003; Shen and Cowan., 2010), dendritic spine formation (e.g. Neuroligin, Ephrins) and synaptogenesis. Increased expression of vesicle associated proteins (e.g., SNAP-25), synaptic vesicle proteins (e.g., synaptophysin, synapsin I) and proteins involved in sodium and calcium-mediated transmitter release occurs during this period. These changes are accompanied by a parallel increase in neurotrophins and neurotransmitters, receptors and ion channels (Sudhof, 2018; Zhong et al., 2020; Rizo and Rosenmund, 2008). Therefore, any alterations in the expression of these proteins (Figure 3) may result in changes of synapse formation, followed by alteration of neuronal networks within the hippocampus.

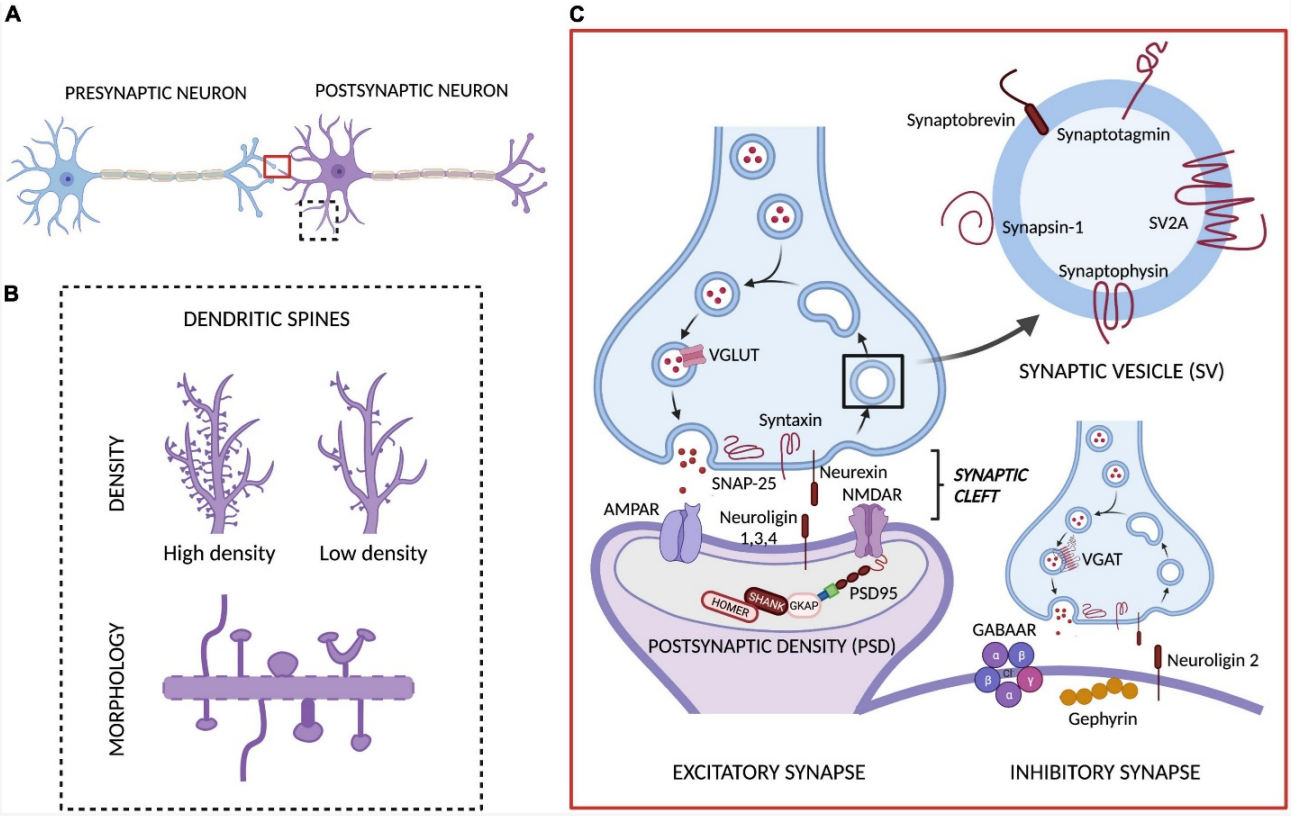


Figure 3. The main structural components of the synapse: (A) a presynaptic and a postsynaptic neuron, separated by a synaptic cleft; (B) the dendritic spines and (C) the proteins involved in synaptic formation and transmission, including synaptic vesicle (SV), presynaptic and postsynaptic proteins present in excitatory and inhibitory synapse. Adapted from Serrano et al., 2022.

The dendritic spine represents the primary site of synaptic activity at the postsynaptic site. A variety of proteins present in the presynaptic terminal and the postsynaptic dendritic spine, are expressed at different times during synaptogenesis, perturbation of which can negatively impact synaptic formation and structure at the macro- and ultra- structural level.

The use of genetically modified mouse models has been widely applied to delineate a host of different proteins involved in the structural development of the hippocampus (Joo et al., 2020). With this approach, changes in neuronal morphology, synapse and network formation in the hippocampus is contrasted in animals lacking this protein vs 'wild type' mice where the protein has been maintained. These comparisons have adopted a variety of techniques, several described below. In KE4, differential expression of proteins identified in these model systems is taken as evidence of altered structure.

However, it is essential to consider the timing of events during development, when their detection is optimal (Hevner, 2007; Garman et al., 2001; Zgraggen et al., 2012). Some macrolevel structural changes may be transient yet still significantly impact downstream events. In the case of knockout models, it is also important to recognize that in most cases, the protein has been removed for the entire lifespan of the animal, in the brain and elsewhere in the body, a scenario distinct from a chemical perturbation.

How it is Measured or Detected

Data in support of this key event have been collected using a wide variety of standard biochemical, molecular, cellular, histological and anatomical methods (e.g., morphometrics, protein quantification using different types of cellular staining, immunohistochemistry, and imaging procedures) at different stages of hippocampus development. Many of methods applied are routine neurohistopathology procedures similar to those recommended in EPA and OECD developmental neurotoxicity guidelines (US EPA, 1998; OCED, 2007). The quantification of cell body and neurite proteins can be carried out by performing immunocytochemistry and automated high content imaging analyses in in vitro and in vivo preparations (Harrill and Mundy, 2011, Meng et al., 2002), Pistollato et al., 2020). Subtle cytoarchitectural features depend on more specialized birth dating procedures and staining techniques. At the micro- and ultra- structural levels, changes in neuronal and glial morphology, alterations in synapse structure, dendritic spine formation (size, shape, number, distribution of head/neck ratios in hippocampal cultures or in vivo studies) and dendritic morphology (branching points, length etc.) can be assessed in Golgi-Cox impregnated neurons (Bongmbaa et al., 2011), two-photon microscopy (Ehrlich et al., 2007), transmission electron (TEM) and flourescent microscopy (Runge et al., 2020; Pchitskaya et al., 2020)

Two-photon time-lapse images can be used to visualise dendrites in GFP-transfected neurons, whereas Golgi Stain is used to measure both dendrites and dendritic spines. A combination of Golgi-Cox and immunofluorescence using confocal microscopy has also been suggested for the visualisation of dendrites in brain slices derived either from rodents or non-human primates (Levine et al., 2013).

Fluorescent markers, such as DiI (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) permits not only the visualisation of detailed dendritic arborizations and spines in cell culture and tissue sections but is also compatible with the quantitative analysis of dendritic spine number (Cheng et al., 2014).

Immunostaining with specific antibodies that recognize presynaptic proteins of excitatory and inhibitory neurons (i.e., vesicular glutamate transporters, vesicular GABA proteins and transporters) and the postsynaptic density protein-95 kDa (PSD-95) can be applied to enumerate synaptse number (Gatto and Broadie, 2010, Akashi et al., 2009). There are commercially available 'synaptogenesis assay kits' that rely on the immunostaining of cells with common synaptic marker proteins such as MAP-2, PSD-95 and synaptophysin. Some other presynaptic (Bassoon) and postsynaptic (ProSAP1/Shank2) markers have been shown to correlate well with the ultrastructural studies in cultured hippocampus primary cells (Grabrucker et al., 2009).

Electron microscopy can also be applied to assess the prevalence of excitatory and inhibitory synapses amongst convergent contacts (Megias et al., 2001). Recently, a high content image analysis based on RNAi screening protocols has been suggested as a useful tool to create imaging algorithm for use in both in vitro and in vivo synaptic punctae analysis (Nieland et al., 2014).

Some of the same techniques used in rodent studies have been applied to postmortem tissue in humans. In addition, non-invasive, structural neuroimaging techniques in living subjects are also widely used in human studies to assess hippocampal volume using voxel-based morphometry (VBM). With this approach, volume of brain regions is measured by drawing 'regions of interest' on images from brain scans obtained from magnetic resonance imaging (MRI) or positron emission tomography (PET) scans calculating the volume enclosed (Mechelli et al., 2005). These imaging techniques can be applied in rodent models (Powell et al., 2009; Hasegawa et al., 2010; Pirko et al., 2005; Pirko and Johnson, 2008).

It is recognized that most of these biochemical, molecular, cellular, histological and anatomical methods (e.g., morphometrics, protein quantification using different types of cellular staining, immunohistochemistry, and imaging procedures) can also be applied to complex in vitro test systems (Pamies et al., 2016; Hartman et al., 2023; Pomeshchik et al., 2020) Human brainspheres or brain organoids could be developed in three-dimensional cell culture, resembling hippocampus at different stages development (Sakaguchi et al., 2015). These new methods, if assessed as robust, reliable and reproducible, would allow measurement of the KE in a human-relevant test system.

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Event: 758: Hippocampal Physiology, Altered

Short Name: Hippocampal Physiology, Altered

Key Event Component

Process	Object	Action
chemical synaptic transmission	synapse	abnormal

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:42 - Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals	KeyEvent
Aop:8 - Upregulation of Thyroid Hormone Catabolism via Activation of Hepatic Nuclear Receptors, and Subsequent Adverse Neurodevelopmental Outcomes in Mammals	KeyEvent
Aop:134 - Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals	KeyEvent
Aop:152 - Interference with thyroid serum binding protein transthyretin and subsequent adverse human neurodevelopmental toxicity	KeyEvent
Aop:300 - Thyroid Receptor Antagonism and Subsequent Adverse Neurodevelopmental Outcomes in Mammals	KeyEvent
Aop:490 - Co-activation of IP3R and RyR leads to socio-economic burden through reduced IQ and non-cholinergic mechanisms	KeyEvent
Aop:458 - AhR activation in the liver leading to Subsequent Adverse Neurodevelopmental Outcomes in Mammals	KeyEvent
Aop:459 - AhR activation in the thyroid leading to Subsequent Adverse Neurodevelopmental Outcomes in Mammals	KeyEvent
Aop:442 - Binding to VGSC during development leads to cognitive function decrease	KeyEvent

Stressors

Name
Propylthiouracil
Iodine deficiency
Methimazole

Biological Context

Level of Biological Organization
Tissue

Organ term

Organ term
brain

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Moderate	NCBI
rat	Rattus norvegicus	High	NCBI
mouse	Mus musculus	High	NCBI

Life Stage Applicability

Life Stage	Evidence
During brain development	High

Sex Applicability

Sex	Evidence
Female	High
Male	High

The majority of evidence for this key event comes from work in rodent species (i.e., rat, mouse). There is a moderate amount of evidence from other species, including humans (Clapp et al., 2012).

Key Event Description

The hippocampus functions as a highly integrated and organized communication and information processing network with millions of interconnections among its constitutive neurons. Neurons in the hippocampus and throughout the brain transmit and receive information largely through chemical transmission across the synaptic cleft, the space where the specialized ending of the presynaptic axon terminus of the transmitting neuron meets the specialized postsynaptic region of the neuron that is receiving that information (Kandell et al., 2012).

During development (see KE 657: Hippocampal anatomy, Altered), as neurons reach their final destination and extend axonal processes, early patterns of electrical synaptic activity emerge in the hippocampus. These are large fields of axonal innervation of broad synaptic target sites that are replaced by more elaborate, but highly targeted and refined axonal projections and synaptic connectivity brought about by activity-dependent synaptic stabilization, pruning, or synapse elimination. This is a classic case of the interaction between physiological and anatomical development, where anatomy develops first, and is ‘reshaped’ by physiological function (Kutsarova et al., 2017).

In the rat, excitatory processes are fully mature in area CA1 of hippocampus within 2 weeks of birth with inhibitory processes lagging begin by several weeks (Muller et al., 1989; Michelson and Lothman, 1988; Harris and Teyler, 1984). In hippocampal slices, inhibitory function in area CA1 field is first seen on postnatal day 5, increasing in strength at postnatal day 12 through 15. In vivo studies fail to detect inhibition until postnatal day 18 with steady increase thereafter to adult levels by postnatal day 28. Synaptic plasticity in the form of long-term potentiation (LTP) is absent in the very young animal, only emerging about postnatal day 14, appearing to require the stability of both excitatory and inhibitory function to be established (Muller et al., 1989; Bekenstein and Lothman, 1991). These features of the maturation of hippocampal physiology are paralleled in dentate gyrus, but as with anatomical indices in the rat, the development of these physiological parameters lag behind the CA1 by about 1 week. As described in structural development in KE4, a very similar pattern of maturation occurs in the human developing hippocampus, but with a trajectory that is largely complete before birth.

How it is Measured or Detected

In animals, synaptic function in the hippocampus has been examined with imaging techniques, but more routinely, electrical field potentials recorded in two subregions of the hippocampus, area CA1 and dentate gyrus, have been assessed in vivo or in vitro. Field potentials recorded in both regions of the hippocampus reflect the summed synaptic response of a population of neurons following direct stimulation of input pathways across a monosynaptic connection. Changes in response amplitude due to chemical perturbations and other stressors (e.g., chemical exposures, nutritional deficits, gene knockouts) is evidence of altered synaptic function. This can be measured in vitro, in vivo, or in hippocampal slices taken from treated animals (Gilbert and Burdette, 1995). The most common physiological measurements used to assess the function of the hippocampus are excitatory synaptic transmission, inhibitory synaptic transmission, and synaptic plasticity in the form of LTP.

Excitatory Synaptic Transmission: Two measures, the excitatory postsynaptic potential (EPSP) and the population spike are derived from the compound field potential at increasing stimulus strengths. The function described by the relationship of current strength (input, I) and evoked response (output, O), the I-O curve is the measure of excitatory synaptic transmission (Gilbert and Burdette, 1995).

Inhibitory Synaptic Transmission: Pairs of stimulus pulses delivered in close temporal proximity are used to probe the integrity of inhibitory synaptic transmission. The response evoked by the second pulse of the pair at brief intervals (<30 msec) arrives during the activation of feedback inhibitory loops in the hippocampus. An alteration in the degree of suppression to the 2nd pulse of the pair reflects altered inhibitory synaptic function (Gilbert and Burdette, 1995).

Long Term Potentiation (LTP): LTP is widely accepted to be a major component of the cellular processes that underlie learning and memory (Malenka and Bear, 2004; Bramham and Messaoudi, 2005). LTP represents, at the synapse and molecular level, the coincident firing of large numbers of neurons that are engaged during a learning event. The persistence of LTP emulates the duration of the memory. Synaptic plasticity in the form of LTP is assessed by delivering trains of high frequency stimulation to induce a prolonged augmentation of synaptic response. Probe stimuli at midrange stimulus strengths are delivered before and after application of LTP-inducing trains. The degree of increase in EPSP and PS amplitude to the probe stimulus after train application, and the duration of the induced synaptic enhancement are metrics of LTP. Additionally, contrasting I-O functions of excitatory synaptic transmission before and after (hours to days) LTP is induced is also a common measure of LTP maintenance (Bramham and Messaoudi, 2005; Kandell et al., 2012; Malenka and Bear, 2004). LTP has been assessed also using in vitro neuronal networks (Odawara et al., 2016; Pre et al., 2022).

Excitatory and inhibitory synaptic currents (EPSCs and IPSCs) can also be measured in single cells, mostly ex vivo within slices of hippocampus using intracellular and patch clamp techniques as described in previous KEs. These same outputs can evaluate the integrity of synaptic transmission and synaptic plasticity.

Synaptic function in the human hippocampus has been assessed using electroencephalography (EEG) and functional neuroimaging techniques (Clapp et al., 2012). EEG is a measure of electrical activity over many brain regions but primarily from the cortex using small flat metal discs (electrodes) placed over the surface of the skull. It is a readily available test that provides evidence of how the brain functions over time. Functional magnetic resonance imaging or functional MRI (fMRI) uses MRI technology to measure brain activity by detecting associated changes in blood flow. This technique relies on the fact that cerebral blood flow and neuronal activation are coupled. Positron emission tomography (PET) is a functional imaging technique that detects pairs of gamma rays emitted indirectly by a radionuclide (tracer) injected into the body (Tietze, 2012; McCarthy, 1995). Like fMRI, PET scans indirectly measure blood flow to different parts of the brain – the higher the blood flow, the greater the activation (McCarthy, 1995). These techniques have been widely applied in clinical and research settings to assess learning and memory in humans and can provide information targeted to hippocampal functionality (McCarthy, 1995; Smith and Jonides, 1997; Willoughby et al., 2014; Wheeler et al., 2015; Gilbert et al., 1998).

Assays of this type are fit for purpose, have been well accepted in the literature, and are reproducible across laboratories. The assay directly measures the key event of altered neurophysiological function.

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List of Adverse Outcomes in this AOP

Event: 402: Cognitive Function, Decreased

Short Name: Cognitive Function, Decreased

Key Event Component

Process	Object	Action
learning or memory		decreased
cognition		decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:42 - Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals	AdverseOutcome
Aop:65 - XX Inhibition of Sodium Iodide Symporter and Subsequent Adverse Neurodevelopmental Outcomes in Mammals	AdverseOutcome
Aop:134 - Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals	AdverseOutcome
Aop:152 - Interference with thyroid serum binding protein transthyretin and subsequent adverse human neurodevelopmental toxicity	AdverseOutcome
Aop:300 - Thyroid Receptor Antagonism and Subsequent Adverse Neurodevelopmental Outcomes in Mammals	AdverseOutcome
Aop:405 - Organo-Phosphate Chemicals induced inhibition of AChE leading to impaired cognitive function	AdverseOutcome
Aop:485 - Thyroid hormone antagonism leading to impaired oligodendrocyte maturation during development and subsequent decreased cognition	AdverseOutcome
Aop:486 - Binding to the extracellular protein laminin leading to decreased cognitive function	AdverseOutcome
Aop:487 - Unknown MIE altering cholesterol metabolism leading to decreased cognition	AdverseOutcome
Aop:488 - Increased reactive oxygen species production leading to decreased cognitive function	AdverseOutcome
Aop:489 - Inhibition of voltage-gated sodium channels leading to decreased cognition	AdverseOutcome
Aop:458 - AhR activation in the liver leading to Subsequent Adverse Neurodevelopmental Outcomes in Mammals	AdverseOutcome
Aop:459 - AhR activation in the thyroid leading to Subsequent Adverse Neurodevelopmental Outcomes in Mammals	AdverseOutcome
Aop:442 - Binding to VGSC during development leads to cognitive function decrease	AdverseOutcome

Stressors

Name
Methimazole
Propylthiouracil
Iodine deficiency

Biological Context

Level of Biological Organization
Individual

Domain of Applicability

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

Basic forms of learning behavior such as habituation have been found in many taxa from worms to humans (Alexander, 1990). More complex cognitive processes such as executive function likely reside only in higher mammalian species such as non-human primates and humans.Basic forms of learning behavior such as habituation have been found in many taxa from worms to humans (Alexander, 1990). More complex cognitive processes such as executive function likely reside only in higher mammalian species such as non-human primates and humans.

Key Event Description

Learning and memory depend upon the coordinated action of different brain regions and neurotransmitter systems constituting functionally integrated neural networks (D'Hooge and DeDeyn, 2001). Among the many brain areas engaged in the acquisition or retrieval of a learned event, the hippocampal-based memory systems have received the most study. The main learning areas and pathways are similar in rodents and primates, including man (Eichenbaum, 2000; Stanton and Spear, 1990; Squire, 2004; Gilbert., 2006).

In humans, the hippocampus is involved in recollection of an event's rich spatial-temporal contexts and distinguished from simple semantic memory which is memory of a list of facts (Burgess et al., 2000). Hemispheric specialization has occurred in humans, with the left hippocampus specializing in verbal and narrative memories (i.e., context-dependent episodic or autobiographical memory) and the right hippocampus, more prominently engaged in visuo-spatial memory (i.e., memory for locations within an environment). The hippocampus is particularly critical for the formation of episodic memory, and autobiographical memory tasks have been developed to specifically probe these functions (Eichenbaun, 2000; Willoughby et al., 2014). In rodents, there is obviously no verbal component in hippocampal memory, but reliance on the hippocampus for spatial, temporal and contextual memory function has been well documented. Spatial memory deficits and fear-based context learning paradigms engage the hippocampus, amygdala, and prefrontal cortex (Eichenbaum, 2000; Shors et al., 2001; Samuels et al., 2011; Vorhees and Williams, 2014; D'Hooge and DeDeyn, 2001; Lynch, 2004; O'Keefe and Nadal, 1978). These tasks are impaired in animals with hippocampal dysfunction (O'Keefe and Nadal, 1978; Morris and Frey, 1987; Gilbert et al., 2016).

How it is Measured or Detected

In rodents, a variety of tests of learning and memory have been used to probe the integrity of hippocampal function. These include tests of spatial learning like the radial arm maze (RAM), the Barnes maze, and most commonly, the Morris water maze (MWM). Tests of novelty such as novel object recognition, and fear-based context learning are also sensitive to hippocampal disruption. Finally, trace fear conditioning which incorporates a temporal component upon traditional amygdala-based fear learning engages the hippocampus. The text below provides brief descriptions of the most commonly used tasks.

1. RAM, Barnes Maze, and MWM are examples of spatial tasks in which animals are required to learn: the location of a food reward (RAM); an escape hole to enter a preferred dark tunnel from a brightly lit open field area (Barnes maze); or a hidden platform submerged below the surface of the water in a large tank of water (MWM) (Vorhees and Williams, 2014).
2. Novel Object Recognition (NOR) and its variants are widely used in neuroscience, although their suitability for safety assessment remains unclear (Vorhees and Williams, 2024). NOR and novel place recognition (NPR) are examples of 'incidental learning' and rely on the dorsal hippocampus. They are simple tasks and are used to probe recognition memory. Two objects are presented to animals in an open field on trial 1, and animals are allowed time to briefly explore them. On trial 2, one object is replaced with a novel object and time spent interacting with the novel object is taken evidence of memory retention (i.e., one of these objects is familiar, the other is novel (Cohen and Stackman, 2015). In novel place recognition, the objects are shifted to a location within the arena. Compared to tests of spatial learning, the learning event is transient, the results often variable, and the test has a very narrow dynamic range.
3. Contextual Fear Conditioning is a hippocampal based learning task in which animals are placed in a novel environment and allowed to explore for several minutes before delivery of an aversive stimulus, typically a mild foot shock. Upon reintroduction to this same environment in the future (typically 24-48 hours after original training), animals will limit their exploration, the context of this chamber being associated with an aversive event (unconditional stimulus, US). The degree of suppression of activity after training is taken as evidence of retention, i.e., memory (Curzon et al., 2009).
4. Trace Fear Conditioning. Standard fear conditioning paradigms require animals to make an association between a neutral conditioning stimulus (CS, e.g., a light or a tone) and an aversive stimulus (US, e.g., a footshock). The unconditioned response (CRUR) that is elicited upon delivery of the footshock US is freezing behavior. With repetition of CS/US delivery, the previously neutral stimulus comes to elicit the freezing response. This type of learning is dependent on the amygdala, a brain region associated with, but distinct from the hippocampus. Introducing a brief delay between presentation of the neutral CS and the aversive US, a trace period, requires the engagement of the amygdala and the hippocampus (Shors et al., 2004).

Most methods used in animals are well established in the published literature, and many have been engaged to evaluate the effects of developmental neurotoxicants. The US EPA and OECD Developmental Neurotoxicity (DNT) Guidelines (OCSPP 870.6300 or OECD 426) both require testing of learning and memory (USEPA, 1998; OECD, 2007). These DNT Guidelines have been deemed valid to identify DNT and adverse neurodevelopmental outcomes (Makris et al., 2009).

A variety of standardized learning and memory tests have been developed for human neuropsychological testing. These include episodic autobiographical memory, word pair recognition memory; object location recognition memory. Some components of these tests have been incorporated in general tests of adult intelligence (IQ) such as the Wechsler Adult Intelligence Scale (WAIS) which calculates four composite scores that examine various domains within an individual's overall cognitive ability: Verbal Comprehension Index (VCI), Perceptual Reasoning Index (PRI), Working Memory Index (WMI), and Processing Speed Index (PSI) (Climie and Rostad, 2011). Modifications have been made and norms developed for incorporating tests of learning and memory in children. Examples of some of these tests include:

1. Rey Osterieth Complex Figure (RCFT) which probes a variety of functions including visuospatial abilities, memory, attention, planning, and working memory (Shin et al., 2006).
2. Children's Auditory Verbal Learning Test (CAVLT) is a free recall of presented word lists that yields measures of Immediate Memory Span, Level of Learning, Immediate Recall, Delayed Recall, Recognition Accuracy, and Total Intrusions. (Lezak 1994; Talley, 1986).
3. Continuous Visual Memory Test (CVMT) measures visual learning and memory. It is a free recall of presented pictures/objects rather than words but that yields similar measures of Immediate Memory Span, Level of Learning, Immediate Recall, Delayed Recall, Recognition Accuracy, and Total Intrusions. (Lezak, 1984; 1994).
4. Story Recall from Wechsler Memory Scale (WMS) Logical Memory Test Battery, a standardized neurospychological test designed to measure memory functions (Lezak, 1994; Talley, 1986).
5. Autobiographical memory (AM) is the recollection of specific personal events in a multifaceted higher order cognitive process. It includes episodic memory- remembering of past events specific in time and place, in contrast to semantic autobiographical memory is the recollection of personal facts, traits, and general knowledge. Episodic AM is associated with greater activation of the hippocampus and a later and more gradual developmental trajectory. Absence of episodic memory in early life (infantile amnesia) is thought to reflect immature hippocampal function (Herold et al., 2015; Fivush, 2011).
6. Staged AM Task. In this version of the AM test, children participate in a staged event involving a tour of the hospital, perform a series of tasks (counting footprints in the hall, identifying objects in wall display, buying lunch, watched a video). It is designed to contain unique event happenings, place, time, visual/sensory/perceptual details. Four to five months later, interviews are conducted using Children's Autobiographical Interview and scored according to standardized scheme (Willoughby et al., 2014).

Regulatory Significance of the AO

A prime example of impairments in cognitive function as the adverse outcome for regulatory action is developmental lead exposure and IQ function in children (Bellinger, 2012). In addition, testing for the impact of chemical exposures on cognitive function, often including spatially-mediated behaviors, is an integral part of both EPA and OECD developmental neurotoxicity guidelines (USEPA, 1998; OECD, 2007).

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Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 2605: Binding to VGSC leads to Altered kinetics of sodium channel

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Binding to VGSC during development leads to cognitive function decrease	adjacent		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Vertebrates	Vertebrates	High	NCBI
Invertebrates	Invertebrates	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

Key Event Relationship Description

VGSCs are critical in generation and conduction of electrical signals in multiple excitable tissues. Various chemicals and agents can interfere with VGSC function through several mechanisms, leading to alterations of VGSC function. The type of alteration depends on how the compound interacts with the VGSC. Hydrophobic anesthetics may bind within the hydrophobic zone in the pore, blocking the channel in the closed state, while hydrophilic anesthetics may bind to the pore on an intracellular site blocking the channel in the inactivation phase. For the latter, high or low dissociation rates affect anesthetic potency in situations of high or low frequency firing, respectively. Other chemicals, like the antiepileptic carbamazepine or the Amyotrophic lateral sclerosis-treatment drug riluzole, bind to the voltage sensors in the channels and thereby shift the voltage dependency of their open/closed configurations. In contrast, toxins like tetrodotoxin (TTX) bind to the extracellular regions of VGSCs, block the passage of ions and cannot be removed by either changing the membrane voltage or the gating of the channel (Eijkelkamp et al. 2012; Catterall 2007). The pyrethroid insecticides also bind to VGSCs but in a manner that slows both activation and deactivation of the gate and results in a more hyperpolarized membrane potential and in higher firing rates (Eijkelkamp et al., 2012; Trainer et al., 1997; O'Reilly et al., 2006; Meyer et al., 2008; Soderlund et al., 2002).

Evidence Supporting this KER

Biological Plausibility

The biological plausibility for this KER is strong. It is a well-accepted fact that ion channels are integral membrane proteins that control the passage of various ions (Na+, K+, Ca2+, Cl−) across lipid membranes in cells. The direction of ion transport through an open ion channel is governed by the electrochemical gradient for the particular ion species across the membrane in question. There is overwhelming evidence that binding of a chemical to a VGSC alters sodium channels kinetics. This is well supported by studies in which individual channel residues are mutated, and these mutations alter the ability of different chemicals to interact with the sodium channel to alter its gating kinetics (e.g. Vais et al., 2000; 2001). The stereospecific nature of effects of many different compounds on VGSC function further supports that specific binding leads to alterations in the kinetics of the channel (Soderlund 1985; Brown et al., 1988; Narahashi 1982).

Empirical Evidence

The empirical evidence for this KER is strong. A wide variety of natural-occurring toxins have been demonstrated to interact with VGSCs and alter function of the channel. These toxins include TTX, the poison in fugu (pufferfish); scorpion and sea anemone toxins, brevotoxins from dinoflagellates, ciguatoxins and some conotoxins from fish-hunting snails. VGSCs possess six or more distinct receptor sites on the VGSC protein, and binding to each of these sites has differential effects on channel kinetics. For example, TTX binds at site 1, irreversibly blocking the pore of the channel and preventing sodium from moving through the channel. Brevotoxin binds to site 5, enhancing activation and preventing inactivation of the channel. The binding of delta conotoxin to site 6 slows channel inactivation (Catterall et al. 2007).

Ample knowledge is also available for synthetic pyrethroid insecticides which bind to the sodium channel α-subunit altering the normal gating kinetics of VGSC. Initial studies attempting to label the specific binding site of pyrethroids were unsuccessful due to the extreme lipophilicity and the modest potency of pyrethroid radioligands. The subsequent development of more potent radioligands demonstrated high affinity saturable binding to sodium channels in the brain. However, the high lipophilicity of pyrethroids still limited the sensitivity of the assay and obscured the identification of the single binding site responsible for pyrethroid action (Soderlund et al., 2002; Trainer et al., 1997). Despite these limitations, there remains overwhelming evidence that binding of pyrethroids to VGSC alters sodium channel kinetics. Mutations in the VGSC in insects alter gating kinetics by decreasing the sensitivity of the channel to pyrethroids, and provide resistance to their toxicity (Vais et al., 2000; 2001). In addition, the effects of pyrethroids are stereospecific where some isomers can interact with and modify channel function while other isomers are unable to bind and have no effect on channel kinetics (Soderlund 1985; Brown et al., 1988; Narahashi 1982). These properties of this class of insecticides on VGSCs are well established in the literature and have been extensively reviewed by Soderlund et al. (2002).

Dose and temporal concordance

Dose-dependent actions of pyrethroids on VGSC kinetics are well documented in the peer-reviewed literature (see Song and Narahashi, 1996, Tabarean and Narahashi, 1998,). On the other hand, temporal concordance for this KER is difficult to measure because of the rapidity (msec) with which chemical binding to the VGSC changes the conformation of the channel and its gating kinetics. However, from the detailed biological understanding of the KER it is clear that binding needs to precede the change in conformation and gating kinetics. In addition, there is clear evidence that the binding to VGSC by some pyrethroids is dependent on particular states of the channel (e.g. open, closed, activated, deactivated). In such cases, modification of the channel kinetics is “use-dependent” , i.e., activation is increased with subsequent stimuli that result in channel opening (Wu et al., 2021; Tabarean and Narahashi, 2001). This characteristic of use-dependence is strong evidence that binding to the channel affects its gating properties.

Uncertainties and Inconsistencies

The fact that binding of chemicals to VGSCs results in altered sodium channel gate kinetics is well accepted and supported by abundant evidence. However, some minor uncertainties can be detected as reported below. Uncertainties in the overall knowledge remain; complete characterization of interactions of chemicals with all α isoforms of the channel, especially in mammals, as well as different subunit combinations have not been conducted, and differences likely exist based on different α and α/β subunit combinations. This is especially true for those channels that might be expressed during development, as the ontogeny of sodium channels is a complex process. Since brain development in both humans and rodents extends from early gestation well into the postnatal period it is not possible to state with certainty which isoform of the sodium channel’s α subunits is preferentially affected.

Quantitative Understanding of the Linkage

Response-response relationship

There are currently no quantitative models that predict the relationship between these KEs. However, it is possible to compute the population of VGSC that are modified by pyrethroid binding, and it has been estimated that less than 1% of the VGSC population (Narahashi et al., 1998) needs to be bound by pyrethroid to disrupt excitability in the neuron (KER2).

Chemicals may bind to VGSCs at various sites leading to different types of changes in the VGSC gate kinetics, and these changes also depend on the affinity of the chemicals to the binding sites (see section above, on KER description). Moreover, there are 9 different types of VGSCs including a complex ontology for the subunits. This complexity currently impedes the characterization of quantitative understanding.

Time-scale

The KER is active within milli-seconds and the upstream event occurs before the downstream event.

Known modulating factors

Species differences are demonstrated for orthologous channels with a high degree of amino acid sequence conservation, which differ in both their functional properties and their sensitivities to pyrethroid insecticides, e.g. with human Nav1.3 channels being not only less sensitive than the rat Nav1.3 channels but also less sensitive than rat Nav1.2 channels (Sun et al., 2009)

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Relationship: 2625: Altered kinetics of sodium channel leads to Disruption in action potential generation

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Binding to VGSC during development leads to cognitive function decrease	adjacent		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Vertebrates	Vertebrates	High	NCBI
Invertebrates	Invertebrates	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

The relationship between activity of VGSC and action potential generation is well described in the literature and highly conserved from low-level phyla (e.g. planarians) to humans, is present in both sexes and throughout development (Smith and Walsh, 2020).

Key Event Relationship Description

Modification of VGSC kinetics may be represented by an alteration in the channel opening or closing. Some modifications, such as blocking by TTX, directly prevent the generation of an action potential. Other VGSC kinetic kinetics may shift the membrane potential required to trigger an action potential. Modification of VGSC kinetics may also be represented by slowing down the activation and inactivation of the channel. This slowing of the timeline increases the channel opening time producing a population of channels that remain open when unmodified channels have closed. A direct consequence of persistent channel opening is depolarization of the membrane to action potential threshold and the induction of repetitive firing of the cell.

However, if the channel is held open for a sufficiently long period, the membrane potential eventually becomes depolarized to the point that generation of action potentials is not possible (depolarization-dependent block). Thus, the effects of disruption VGSC kinetics on the action potential are qualitatively different based on the time the channel remains open and this can be measured electrophysiologically. A limited chemically-induced increase in channel opening will lead to repetitive firing while a prolonged opening blocks action potential generation (Shafer et al., 2005).

Evidence Supporting this KER

Biological Plausibility

The biological plausibility of KER2 (*Altered kinetics of sodium channel leads to Disruption in action potential generation*) is **strong**. The rising phase of an action potential is caused by the opening of voltage-gated sodium channels. These ion channels are activated once the cell's membrane potential reaches a threshold and open immediately. The electrochemical gradients drive sodium into the cell causing a strong and abrupt depolarization characteristic of an action potential. The falling phase of the action potential is caused by the inactivation of the VGSCs stopping further sodium influx, and the opening of voltage-gated potassium channels. As K+ concentrations inside the cell are very high, channels open and the current flow out serves to restore the membrane potential toward its resting state. However, the efflux of K+ ions is large leading to a hyperpolarisation (undershoot phase) of the membrane potential. Ultimately the voltage-gated K+ channels close and the membrane potential returns to its resting state. This is very well-established textbook knowledge. While it is well accepted that various combinations of channel types in a cell can give rise to differences in the shape and time course of the action potential, the underlying biological principles and relationships between VGSC and action potentials are maintained. Expression of VGSCs is spatially and temporally dependent and have differential expression during CNS development. It is clear that as in the adult, binding to VGSC isoforms will also disrupt the channel gating kinetics and action potential generation in the developing brain (see reviews by Shafer et al., 2005; Soderlund et al., 2002).

Empirical Evidence

The empirical evidence of KER2 is strong. As described in KE2, natural toxins like TTX bind to VGSC and block all electrical activity including action potentials. The relationship of VGSC and action potential generation has been widely demonstrated with a variety of other stressors (e.g., local anesthetics, anticonvulsants and other pharmacological agents (Hwang et al., 2020; Lee et al., 2015). A large body of literature on pyrethroids insecticides has confirmed their ability to alter action potential firing in both insect and mammalian peripheral and central in vitro and in vivo preparations. These studies have been extensively reviewed (Soderlund et al., 2002; Narahashi et al., 1998; Bloomquist, 1996).

Dose and temporal concordance

Although it is well established that altered kinetics of VGSC lead to disruption of action potentials, due to the "all or none" firing characteristics of neuronal action potentials, it is difficult to demonstrate dose-concordance for this specific KER. However, there are examples in the literature showing dose concordance between concentration of pyrethroid insecticides and action potential generation. For deltamethrin and permethrin, changes in VGSC kinetics and disruption of the action potential are reported in vitro at concentration between 0.01 to 1 mM, in hippocampal or neocortical neurons from postnatal day 2–4 pups (Meyer et al., 2008; Cao et al., 2011). Similarly, Song and Narahashi (1995; 1996) demonstrated dose concordance for tetramethrin. As described here and in KER1, there is substantial evidence of dose-related effects of pyrethroid insecticides.

Similar to the temporal concordance between binding of compound to the VGSC and altered kinetics (KER1), alteration of VGSC kinetics and membrane excitability/action potential generation also occur very quickly. Thus, temporal concordance is difficult to demonstrate directly using experimental approaches. As mentioned, pyrethroid effects have been described as "use-dependent". As such, increased modification of the channel with repeated depolarizations results in further disruption of action potentials.

Uncertainties and Inconsistencies

Evidence supporting this KER is derived nearly entirely from in vitro experiments, as it is not possible to measure directly sodium channel function in vivo, only proxies of it. However, in vivo recordings of action potentials demonstrate repeated firing in both mammalian and non-mammalian species, supporting that the KER relationship exists across species and in intact nervous systems. Additional uncertainty exists due to the diversity of different sodium channel subunits and understanding their role in the action potential. Thus, the exact compositions of sensitive channels are not characterized. With respect to temporal relationships, different pyrethroid compounds exhibit differing levels of use dependence (Soderlund, 2010), which can be influenced by channel type. However, the level of evidence supporting this KER in the peer-reviewed literature is abundant and the confidence in this KER is high.

Quantitative Understanding of the Linkage

Generation of action potentials and the roles of different ion channels in action potential generation and propagation are well understood, and described by the Hodgkin-Huxley model, so theoretically, a quantitative model could be constructed that incorporates alterations in VGSC kinetics and links to action potential generation. It has been estimated than an increased in opening time of a small percentage of VGSCs (< 1% of the VGSCs in a neuron) is all that is required to trigger repetitive firing of that neuron, and an accelerated cycling of the naïve VGSC to cycle through their resting/open/inactivation stages (Narahashi, 1996). Computational models of VGSC conductance and action potential generation have been published (Santha-Kumar et al., 2005). These models exemplify both dose and temporal concordance between these two KEs.

Response-response relationship

The relationship between alteration of VGSC kinetics and action potential generation has been modeled in neuroblastoma cells for tetramethrin (Mohan et al., 2006; Molnar and Hickman, 2014). However, the extent to which this model has been extended to other pyrethroids is not clear.

Time-scale

The KE channel opening lasts micro-seconds and modification by compounds occurs quickly, but in the case of state dependence, can be exacerbated with repeated depolarization. Action potentials typically last less than a millisecond under normal biological conditions. Modification of the VGSC by pyrethroids can result in repeated firing of action potentials that occur for hundreds of milliseconds (e.g., Song and Narahashi, 1996). Thus, KER happens within milliseconds to microsecond time-scale.

Known modulating factors

As noted above, the composition of different VGSC channel subunits, as well as compositions of voltage-gated potassium and calcium channels in the cell can influence the overall shape and timing of the action potential. This includes changes that might be the result of developmentally specific expression of channels and subunits.

Known Feedforward/Feedback loops influencing this KER

As described above, the state-dependent interaction of pyrethroids can result in exacerbation of effects with repeated depolarizations. When VGSC inactivation occurs at for short intervals, action potentials are fired repetitively. Such is the case for permethrin and other Type I pyrethroids. By contrast, pyrethroids (type II) prolonged VGSC inactivation for a longer period of time, depolarizing the membrane potential to the point that action potentials can no longer be generated - depolarization-dependent block (Shafer et al., 2005).

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Relationship: 3242: Disruption in action potential generation leads to neurotrasmission in development

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Binding to VGSC during development leads to cognitive function decrease	adjacent		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Vertebrates	Vertebrates	High	NCBI
Invertebrates	Invertebrates	High	NCBI

Sex Applicability

Sex	Evidence
Male	High
Female	High

Male, females, all life stages, starting from foetal stage (Smith and Walsh 2020).

Key Event Relationship Description

Stimulation of neurons by neurotransmitters or sensory input activates the opening of different ion channels and permits current flow across the membrane. As ion currents move across the membrane, the membrane potential is changed. Depending on the ion channel, this change in membrane potential can be either excitatory to depolarize, or inhibitory to hyperpolarize the cell. Neurons integrate the barrage of both excitatory and inhibitory signals they receive. When this integration leads to a net sum depolarization, voltage gated sodium channels open and an action potential is triggered. The action potential through a series of successive openings of additional VGSCs, allows the transmission of the electrical impulses to move along the length of the axon to the nerve terminal. The synapse describes the location where the presynaptic nerve terminal meets the postsynaptic cell. The postsynaptic cell can be another neuron, muscle or gland. At the synapse the electrical signal at the presynaptic terminal is transduced to a chemical signal to span the spatial gap and communicate information from one cell to the next. On arrival of the depolarizing action potential (AP) at the presynaptic terminal, voltage gated calcium channels are activated and vesicles containing chemical neurotransmitters are released into the synaptic cleft – the space between the neurons. The frequency and duration of the action potentials determines how many neurotransmitter vesicles are released. The neurotransmitters act on the postsynaptic cell by interaction with neurotransmitter-specific receptors that depolarize or hyperpolarize the membrane of the receiving cell. This transduction of electrical to chemical and back again to electrical signaling across neurons is the basis of neurotransmission. This sequence of events is portrayed in Figure 4.

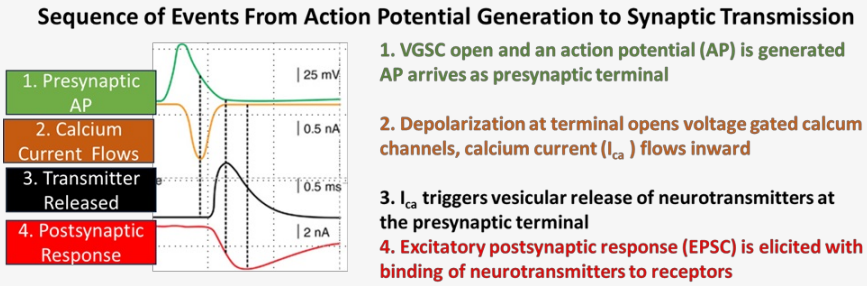

Figure 4. Sequence of Events from action potential generation to synaptic transmission.

It is well established that neurotransmission can be disrupted through several different mechanisms, including disruptions of ion channels, release machinery, post-synaptic response and disruption of neurotransmitter re-uptake or degradation (Atchison, 1988; Vester and Caudle, 2016). It is also well accepted that neurotransmission occurs in the mature and developing brain and can be similarly disrupted by the same mechanisms.

Evidence Supporting this KER

The evidence supporting this KER a well-established tenant of neurobiology (Foundations of Neuroscience by Casey Henley;https://openbooks.lib.msu.edu/neuroscience/chapter/drug-and-toxin-effects/ ; more detailed in Cellular and Molecular Neurophysiology There is abundant evidence that disruption of action potentials leads to altered neurotransmission by drugs and environmental agents, including VGSC blockers (Meng et al., 2016; Shafer et al., 2008; Hossain et al., 2008; for review, see Soderlund et al., 2002). There are also numerous examples of peer-reviewed studies demonstrating alterations in action potential activity leading to altered neurotransmission during development (Čechová and Šlamberová, 2021; Latchney et al., 2021).

Biological Plausibility

The process of disruption of action potentials leading to changes in neurotransmission represents a very well-established principle of neurobiology that is widely described in the published

literature and basic neuroscience textbooks. This process is the basis of routine neurophysiological studies investigating the development, function and disturbance of neuronal networks. It is not only biologically plausible that alterations in action potential shape, duration and patterns could lead to altered neurotransmission, but also that this occurs in adult and developing nervous systems.

Empirical Evidence

The empirical evidence of KER3 is strong. There is abundant empirical evidence in the published literature supporting the basic biology underlying this KER. A variety of insults including chemical insults can alter action potential generation and impair synaptic transmission (e.g. Seabrook et al., 1989; Joy et al., 1990; Hong et al., 1986; Gilbert et al., 1989. Eells and Dubocovich, 1988; Hossain et al., 2008; Shafer et al., 2008). These data have been generated in a wide variety of different models from insects to mammalian models, including embryonic neurons and adult neuronal preparations. For a more detailed explanation, and examples of chemicals and mechanisms leading to altered neurotransmission, the readers are referred to https://openbooks.lib.msu.edu/neuroscience/chapter/drug-and-toxin-effects/

Dose and temporal concordance

Because of the challenges of measuring this KER, dose concordance is not well established. However, there is no indication of discontinuity between dose levels that alter action potential activity and synaptic transmission. In addition, dose-concordance is observed at both earlier and later KERs, indicating that it likely is maintained for this KER.

Action potential stimulation of neurotransmission occurs in a very rapid millisecond time scale. There is strong evidence for temporal concordance between alterations in action potential activity and changes in neurotransmission. The sequence of events from presynaptic action potential generation and postsynaptic response as depicted above in Figure 4, clearly demonstrates the time concordance between these two KEs.

Uncertainties and Inconsistencies

The biological processes that regulate the generation and propagation of action potential and neuronal transmission are very well known and changes in this KER are well documented for chemical insults. This KER is supported by both in vitro and in vivo data.

The literature directly demonstrating the relationship between action potential generation and neurotransmission during development is less robust. However, given the fundamental properties of neurotransmission that exist in both mature and developing nervous system and the extensive literature of chemical stressors derived from a wide variety of preparations of varying ages, this uncertainty is small.

Quantitative Understanding of the Linkage

Currently, quantitative models for this KER were not found in the peer-reviewed literature.

Response-response relationship

The overall relationship between action potential firing leading to release of neurotransmitter release and a response in the post-synaptic cell is well established in neurobiology. One simple example is the firing of a motor neuron, leading to release of acetylcholine, followed by muscle contraction. The sequence of events from presynaptic action potential generation and postsynaptic response as depicted above in Figure 1, clearly demonstrates the response-response concordance between these two KEs. The precise form of the response-response relationships in terms of the either excitation or inhibition and strength of that effect is dependent on the neuron type and its location and function within the nervous system.

Time-scale

The KER is active within milli-seconds and the upstream event occurs before the downstream events (see Figure 4 above).

Known modulating factors

The description of the KER provided here is a generic description, but the basic biology described is maintained across species, developmental stage, brain regions and sex. There are a number of factors that can modulate this relationship, including, but not limited to, temperature; region/pathway/neuronal subtype, type of synaptic structure, age of the animal and preceding activity at that synapse.

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Relationship: 3243: neurotrasmission in development leads to Hippocampal anatomy, Altered

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Binding to VGSC during development leads to cognitive function decrease	adjacent		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Vertebrates	Vertebrates	High	NCBI

Term	Scientific Term	Evidence	Links
Invertebrates	Invertebrates	High	NCBI
rat	Rattus norvegicus	Moderate	NCBI
mouse	Mus musculus	Moderate	NCBI
human	Homo sapiens	Not Specified	NCBI

Life Stage Applicability

Life Stage	Evidence
Development	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

This KER is supported from rodent models, in which hippocampal brain slices have been studied ex vivo. Despite the hippocampus is structurally quite different among mammals, birds and reptiles, its function in spatial memory is highly conserved (Striedter, 2016). This suggests, with some uncertainty, that this KER is also applicable to multiple species.

Activity-dependent alterations in brain connectivity and synaptic structure occurs in males and females, in all mammals, at all life stages, and is especially prominent during development. Structural remodeling also occurs in the non-mammalian species.

Key Event Relationship Description

It is well established that neurons extend and retract their pre and postsynaptic processes dependent on the level of neuronal activation (Andrae et a., 2014). These growth processes determine the basic shape of a neuron and its regions of afferent and efferent connections, processes critical during brain development. Neural systems encode information structurally via the wiring between neurons and this wiring is modulated by the electrical activity in both the developing and adult nervous systems. Establishment of synaptic connectivity begins as a diffuse process that is refined in an activity-dependent manner during development (Pan and Monje, 2020). Disruption of the formation of precise neural circuits during the prenatal and perinatal stages of brain development may underlie neurodevelopmental disorders.

At birth, an infant’s brain contains more neurons than present in the adult. As the child grows, experiences strengthen circuits that prove more relevant and weaken others. This process of overgrowth followed by selective activity-dependent elimination is key to forming an adaptive brain, with waves of neuronal cell death and dramatic reduction in connecting axonal fibers occurring during development (Anosike et al., 2023). The process whereby a subset of synapses is removed, while others are maintained is called synaptic pruning. It is a fundamental property of the mammalian CNS, it occurs in response to changes in neural activity, and it is most prominent in the developing nervous system (Faust et al., 2021). The lack of activity at the majority of synapses on a neuron can lead to the eventual death of that cell. As such, interference with electrical signaling during development can certainly influence connectivity of the developing brain.

The activity-dependent processes alter both the structure of the axonal bouton of presynaptic and the dendritic spine of the postsynaptic neuron. Spines can change in shape, volume, density, and location. Overall activity levels can increase or decrease spine number, dendritic branches can be expanded or eliminated, axons removed or redirected to novel destinations based on the level of activity with the synaptic network. Denervation inducing a total lack of neuronal activity can induce axonal growth and expansion to other areas.

Spontaneous neurotransmitter release plays an important role in shaping neuronal morphology as well as modulating the properties of newly forming synaptic connections in the brain (Andreae and Burrone, 2018). Excessive or insufficient neurotransmission during critical windows of development can affect the complexity of the connectivity within pre- and post-synaptic neurons, leading to altered synaptic density and connectivity. The delicate balance between excitatory (glutamatergic) and inhibitory (GABAergic) neurotransmission shapes brain circuitry, and when perturbed, it can lead to abnormal network activity (Cherubini et al., 2021). This has been widely studied in the hippocampus.

There are two types of structural remodelling, one occurring on fast (minutes to hours), the other on a more protracted (hours, days, weeks) timescale. Activity-dependent remodelling can occur at ultrastructural, network, and regional levels, and in the developing nervous system as well as the mature brain (see review by Fauth and Tetzlaff, 2016). Changes in neuronal architecture driven by activity are known to occur in all brain areas studied to date including the hippocampus.

Evidence Supporting this KER

Biological Plausibility

The biological plausibility of altered neurotransmission during the development and further impairment of hippocampal anatomy is strong. Extensive evidence supports the notion that disruption of neurotransmission during development can induce micro-structural morphological changes in the hippocampus. This can occur due to the effect of various factors such as genetic mutations, brain damage, environmental toxins, and stress during vulnerable periods of brain development.

Impaired synaptic transmission may occur at pre- or postsynaptic level and involves disruption of the normal functioning of neurotransmitters, their receptors, or scaffolding proteins. The strength of the synaptic transmission can be modulated by the amount of neurotransmitter released, the number of receptors on the postsynaptic cell, and their sensitivity to the neurotransmitter due to alterations in the number and conductance of postsynaptic receptors (Graziane and Dong, 2022; Hestrin, 2015). In case of presynaptic dysfunction, either too much or too little neurotransmitter may be released into the synaptic cleft, whereas in postsynaptic dysfunction, the postsynaptic neuron may not respond adequately to that neurotransmitter. In both cases, the altered synaptic transmission may have pre- or postsynaptic morphological consequences, including e.g. number of docked vesicles at the nerve terminal, or the number, density and morphology of dendrite spines. These changes may affect the structure and function of neural circuits and may underlie behavioral deficits (Bonnycastle et al., 2021).

Empirical Evidence

The evidence supporting this KER is considered moderate. Neurites of single cells in culture grow and retract depending on the level of neuronal activation (Cohan and Kater, 1986). Pharmacological block of action potentials by saxitoxin curtails synaptic transmission in PC12 and SH-SY5Y cell lines and inhibits neurite outgrowth (O’Neill et al., 2017). Electrical stimulation to activate synaptic transmission induces rapid input-specific changes in dendritic structure; however, these changes are reversed when neurotransmission is blocked (Kirov and Harris, 1999). These phenomena have been demonstrated in developing hippocampal cultures, dissociated neuronal cultures, organotypic slices and in intact organisms. The number, volume, density, and shape of dendritic spines can all be altered with electrical stimulation. Spine growth is input specific, occurs only close to activated parts of the dendrite, and can be eliminated by blocking synaptic transmission at the postsynaptic receptor. Chronic blockade of neuronal activity leads to the reversible growth of dendritic spines in the hippocampus, while persistent activity-dependent changes in spine structure contributes to the development and refinement of neural circuitry (Maletic-Savatic et al., 1999; Kirov and Harris, 1999).

Cultured cortical neurons deprived of action potentials by an extended period of tetrodotoxin (TTX) treatment initially showed a marked increase in size and frequency of mEPSCs, indicating a rise in the postsynaptic response to glutamate. Morphologically, these neurons retracted their dendrites, lost dendritic spines, and eventually degenerated over a period of 1–2 weeks. Neuronal morphological deterioration was prevented by blockade of glutamatergic AMPA receptors (Fishbein and Segal, 2007). As such, the block of action potential generation and consequent neurotransmission impairment can lead to altered morphology by both direct and indirect means.

Both higher and lower levels of activity can drive structural change in positive and negative directions, at ultrastructural and macrostructural scales. For example, unrelated to neuronal damage, elevated levels of electrical activity accompanying epilepsy reduce spine number (Geineisman et al., 1990). Sensory deprivation leading to lower activity levels in neurons can increase the number of newly formed spines. Some examples include monocular deprivation in the mouse that eliminates electrical activity in visual cortex neurons in one hemisphere, doubles the number of newly formed spines in the binocular region of the same hemisphere (Hofer et al., 2009). Similarly, trimming the whiskers of rats to eliminate excitation of somatosensory neurons leads to an increased number of spines and an outgrowth of dendritic trees into the barrel field of the cortex (Vees et al., 1998). With a delay of several days, axons from the neighboring neurons, unaffected by the deprivation, grow toward the deprived region. These adjacent neurons, although unaffected by the deprivation, experience altered activity levels, triggering their axonal growth. In both visual and somatosensory models, structural plasticity is most pronounced during specific limited time windows in brain development.

In the hippocampus, electrical stimulation of afferents alters spine number and morphology of pyramidal and granule cell neurons in vitro and in vivo (Kirov et al., 2004; Kirov and Harris, 1999; Geineisman et al., 1990; Maletoc-Savatic et al., 1999) and increases neurogenesis in the adult dentate gyrus (Chun et al., 2006; 2009; Gilbert et al., 2020).

Activity-dependent structural changes in connectivity have been amply documented in adult networks and in the developing brain. It is widely accepted that activity-dependent morphological growth and restructuring is paramount in development. Specific patterns of change may be different in the mature versus the developing nervous system, but that activity is the trigger of structural change is not in doubt.

Dose and temporal concordance - Essentiality

The evidence is clear that synapse formation, synapse pruning, and the establishment and fine tuning of neural circuits in the developing brain requires neurotransmission. As such, alterations in neurotransmission during development drive changes in post-synaptic structure in the hippocampus.

Uncertainties and Inconsistencies

Changes in connectivity have not been directly linked to electrical activity per se, but neuronal activity is essential to trigger complex molecular signaling cascades, which mediate to the corresponding structural changes. In many cases, calcium signaling is used as a surrogate measure of electrical activity at the synapse as postsynaptic calcium level is largely dictated by neuronal activity. However, the detailed relation between calcium, activity, and spine dynamics is more complex, as the calcium level is also regulated by other signals such as neurotrophins and adhesion molecules (Stoop and Poo, 1996; Bixby et al., 1994).

Quantitative Understanding of the Linkage

Several theoretical and computational models of structural plasticity exist and range from simple single neuron connections to complex neural networks. Both dynamics of dendritic spines on a brief timescale to longer timelines of structural connectivity have been described and reviewed by Fauth and Telzlaff (2016).

Response-response relationship

The connection between activity and structural change is well documented and the nature of the structural alteration can be growth and stabilization or destabilization and elimination at the synaptic level. Elimination of entire neurons can occur in complete absence of activity, while at the same time, absence of activity can trigger growth of adjacent neurons to a denervated site.

Time-scale

Seconds to minutes, hours to days, days to weeks, mature and immature organisms

Known modulating factors

Although activity dependent alterations in synaptic structure occur in both males and females, hormones can modulate their extent, serving to stabilize connections in some cases, while destabilizing and eliminating connections in others. Other hormonal systems, notably glucocorticoids can modulate activity-dependent structural change.

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Relationship: 749: Hippocampal anatomy, Altered leads to Hippocampal Physiology, Altered

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals	adjacent	Moderate	Low
Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals	adjacent	Moderate	Low
Thyroid Receptor Antagonism and Subsequent Adverse Neurodevelopmental Outcomes in Mammals	adjacent	Moderate	Low
Upregulation of Thyroid Hormone Catabolism via Activation of Hepatic Nuclear Receptors, and Subsequent Adverse Neurodevelopmental Outcomes in Mammals	adjacent		
Binding to VGSC during development leads to cognitive function decrease	adjacent		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	Moderate	NCBI
mouse	Mus musculus	Moderate	NCBI
human	Homo sapiens	Not Specified	NCBI

Life Stage Applicability

Life Stage	Evidence
During brain development	High

Sex Applicability

Sex	Evidence

Sex	Evidence
Male	High
Female	High

The majority of data in support of this KER is from rodent models, supported by limited evidence in humans. The evolutionary conservation of hippocampal anatomy in mammals, birds, and reptiles (see Hevner, 2016; Streidter, 2015) suggests, with some uncertainty, that this KER is also applicable to multiple species.

Key Event Relationship Description

The hippocampus is a highly integrated and organized communication and information processing network with millions of interconnections among its constitutive neurons (see Andersen et al, 2006). Neuronal spines are the primary site of action for synaptic interface between neurons. Although difficult to measure due to their small size, large number and variable shapes, changes in the frequency and structure of dendritic spines of hippocampal neurons has dramatic effects on synaptic physiology and plasticity (Harris et al., 1984). Anatomical integrity at a more macro-level is also essential for physiological function. The connectivity of axons emanating from one set of cells that synapse on the dendrites of the receiving cells must be intact for effective communication between neurons to be possible. Synaptogenesis is a critical step for neurons to be integrated into neural networks during development. Changes in the placement of cells within the network due to delays or alterations in neuronal migration, the absence of a full proliferation of dendritic arbors and spines upon which synaptic contacts are made, and the lagging of transmission of electrical impulses (e.g., due to insufficient myelination) will independently and cumulatively impair synaptic function.

It is also well documented that at the molecular level (mainly based on studies in genetically modified mouse models summarized in Table XX) that disruption in the expression of pre- and post-synaptic proteins involved in the presynaptic vesicular docking, fusion or release of neurotransmitters impair stabilization of synapses during development. This ultimately alters cytoarchitecture at the macro and ultrastructural level and leads to modification in the physiological integrity of neural circuits in the mature brain. The functional impact of these macro- and micro- alterations hippocampus at the structural level are most often revealed by deficits in neurotransmission and activity-dependent plasticity.

Evidence Supporting this KER

The weight of evidence supporting the relationship between structural abnormalities in brain induced and altered synaptic function is moderate. There is no doubt that altered structure can lead to altered function. Many examples from knock out models, genetic mutations, prenatal alcohol, nutritional deficits demonstrate a correlative link between altered structure and impaired synaptic function within the hippocampus (Gil-Mohapel et al., 2010; Berman and Hannigan, 2000; Grant et al., 1992; Palop et al., 2010; Ieraci and Herrera, 2007). However, the scientific understanding of the causative and quantitative relationship between the two KEs is incomplete.

Biological Plausibility

The biological plausibility of alterations in hippocampal structure impacting synaptic function and plasticity in the brain is **strong**. Because synaptic transmission in the hippocampus relies on the integrity of contacts and the reliability of electrical and chemical transmission between pre- and post-synaptic neurons, it is well accepted that interference on the anatomical levels will largely impact the functional output on the neurophysiological level (Knowles, 1992; Schultz and Engelhardt, 2014).

Extensive research has provided substantial data on the characteristics supporting a direct link between alterations in neuronal anatomy (axon and dendritic spines morphology, shape and density, vesicular proteins and release, synaptogenesis and neuronal network formation) and neurotransmission, particularly in the context of activity-dependent changes in synaptic strength (synaptic plasticity), best exemplified in the phenomenon of long-term potentiation (LTP). For instance, spine structure is closely linked to synapse function, as the size of spine heads scales with synaptic strength (Matsuzaki et al., 2001; Noguchi et al., 2011). Moreover, the shape and number of spines can be modified by the induction of synaptic plasticity (Matsuzaki et al., 2001; Tønnesen et al., 2014; Zhou et al., 2004). These anatomical alterations in hippocampus lead to changes in the electrophysiological properties of this brain region. Specifically, they serve as physiological readouts of hippocampal function at the synaptic level. The most common physiological readouts were revealed as impairments in basal neurotransmission, synaptic inhibition, and synaptic plasticity (LTP and LTD) (Schnell et al.,2002; Ehrlich & Malinow, 2004; Schmeisser et al., 20129). As detailed in KER4, these same activity-dependent processes are invoked as mechanistic underpinnings for how neuronal activity impacts structure, especially in the developing brain.

Empirical Evidence

Empirical support for this KER is rated as **moderate**. There is no doubt that alterations of the structure of the hippocampus can lead to alterations of its function. Both in vivo and in vitro studies have demonstrated changes in glial and neuronal cell number or morphology impact physiological function in the hippocampus. Alterations in neurite number, length and complexity have been documented in hippocampal slice cultures with corresponding changes in synaptic function (Hosokawa et al., 1995). Chemical stressors (e.g., prenatal alcohol, developmental Pb exposure, hypoxia), nutritional deficits, and selective lesion models demonstrate a correlative link between altered structure and impaired synaptic function within the hippocampus (Gil-Mohapel et al., 2010; Berman and Hannigan, 2000; Palop et al., 2010; Ieraci and Herrera, 2007). Numerous examples of a direct linkage between hippocampal anatomy and hippocampal physiology are evident in knock out or transgenic mouse models (e.g., Lessman et al., 2011), a few of which are detailed below.

Mutations of the tyrosine kinase gene Fyn during development increased the number of neurons in the dentate gyrus and CA subfields of the hippocampus. Fyn mutant mice also exhibited impairments in long-term potentiation in hippocampal CA1 whereas two other forms of short-term plasticity remained intact (Grant et al., 1992).

Neuroregluin-2 (NRG2) is a growth factor that is highly expressed in the hippocampal dentate gyrus where it contributes to synaptogenesis of newborn granule cells. In hippocampal slice cultures, inducible microRNA targeting strategies have shown that suppression of NRG2 reduced synaptogenesis of inhibitory neurons and impaired dendritic outgrowth and maturation of glutamatergic synapses. These anatomical alterations were accompanied by reductions in the amplitude of excitatory synaptic currents. The magnitude of the impairment was dependent on the timing of the infection and could be eliminated with overexpression of NRG2 in this in vitro model (Lee et al., 2015).

Brain-derived neurotrophic factor (BDNF) activation of CREB-activated gene expression plays a documented role in hippocampal synaptogenesis, dendrite formation, and synaptic plasticity in the developing and adult nervous systems (Lessmann et al., 2011; Panja and Bramham, 2014). Jacob is a protein that translocates to the nucleus upon activation of BDNF-dependent pathways and is involved in both neuronal plasticity and neurodegeneration. Hippocampal neurons in culture derived from Jacob/Nsmf knockout mice exhibit shorter neurites with reduced branching and fewer synaptic contacts. This effect was specific to hippocampal neurons, as cortical cells derived from the same animals did not display these abnormalities. In vivo, these animals exhibited a reduction of dendritic complexity of CA1 neurons, lower number of branches, and decreased spine density. Deficits in synaptic plasticity in the form of LTP accompanied these structural impairments (Spilker et al., 2016).

Knockout of PSD-95 (a post-synaptic protein which regulates AMPA-R trafficking and synaptic maturation) impaired long-term depression in CA1 neurons and decreased synaptic strength. Loss of PSD-95 thwarted the developmental increase in the number of functional AMPA-Rs expressing synapsis and prevented developmental changes in spine density and morphology (decreased spine size, a larger number of transient spines that were less stable), arresting synapses in a more immature state (Ehrlich et al., 2007). However, overexpression of PSD-95 increased synaptic strength (by enhancing LTD (Schnell et al.,2002; Ehrlich & Malinow, 2004).

IKK/NF-κB signaling is critically involved in synapse formation and spine maturation in the adult brain. IKK/NF-κB blockade in hippocampus of mutant animals was associated with reduced levels of mature spines and postsynaptic proteins (PSD95, SAP97, GluA1), and AMPAR-mediated basal synaptic transmission was suppressed. Exogenous Igf2 (IKK/NF-κB target) was able to restore synapse density and promote spine maturation (Schmeisser et al., 2012).

In Alzheimer's Disease, amyloid-β protein accumulates in the hippocampus and leads to the formation of amyloid plaques, neuritic dystrophy and aberrant sprouting of axon terminals of the hippocampus. In a developmental germ-line knockout mouse model, high levels of amyloid-β induced aberrant neuronal network excitability and altered innervation of inhibitory interneurons. Deficits in hippocampal plasticity were seen in the dentate gyrus without change in basal levels of synaptic transmission. In contrast, in area CA1, synaptic transmission was impaired while measures of synaptic plasticity remained intact (Palop et al., 2007).

Other evidence for a direct linkage between hippocampal anatomy and hippocampal physiology comes from the area of adult neurogenesis. The neurogenesis process refers to the acquisition of new neurons on the hippocampus of the adult brain and is associated with enhanced hippocampal synaptic function and learning ability (Deng et al., 2010). Manipulations such as caloric restriction, exercise and hormones can enhance neurogenesis and increase synaptic transmission and plasticity (Kapoor et al., 2015; Trivino-Paredes et al., 2016; Deng et al., 2010). A reciprocal relationship also exists whereby increases in hippocampal neural activity serves to increase neurogenesis (Bruel-Jungerman et al., 2007, Bruel-Jungerman et al., 2009, Kameda et al., 2012). Manipulations that decrease hippocampal neurogenesis including exposure to antidepressants, hormone disruption, radiation, genetic ablation, stress, and alcohol are also associated with impaired synaptic function (Herrera et al., 2003; Saxe et al., 2006; Gilbert et al., 2016; Montero-Pedrazuela et al., 2006; Gil-Mohapel et al., 2010).

Temporal Evidence

The temporal nature of this KER is developmental (Seed et al., 2005). This has been demonstrated in multiple studies. A few examples detailed above defined critical periods for the manipulation that alters the structural development of the hippocampus that persists to adulthood to disrupt the synaptic physiology measured in the hippocampus in adulthood (Lee et al., 2015; Grant et al., 1992). A more limited number of 'rescue' experiments have been reported. As described above in Empirical Evidence supporting this KER, Lee et al (2015), using an in vitro model, demonstrated impaired synaptogenesis that was dependent on the timing of the infection and could be eliminated with overexpression of NRG2. In Spliker et al (2016), BDNF application rescued the morphological deficits in hippocampal pyramidal neurons from Jacob/Nsmf mice. Knockout of PSD-95 which altered spine density and morphology resulting in impaired long-term depression in CA1 neurons (decreased synaptic strength) that was rescued by overexpression of PSD-95 (Schnell et al.,2002; Ehrlich & Malinow, 2004). Similarly, in Schmeisser et al (2019) IKK/NF- B blockade in hippocampus of mutant animals reduced levels of mature spines and postsynaptic proteins (PSD95, SAP97, GluA1) and AMPAR-mediated basal synaptic transmission which was restored by exogenous application of Igf2 (IKK/NF- B target).

Dose-Response Evidence

Dose-response data is lacking for this KER. For future research, it is critical to generate data in which the upstream KE is modulated in a 'dose-response' manner to better support the causative relationship.

Uncertainties and Inconsistencies

There are no inconsistencies in this KER, but there are uncertainties. Although several examples are evident to demonstrate direct linkages between alterations in hippocampal anatomy and disruptions in hippocampal physiology, there is not a common cellular mechanism, anatomical insult, or signature pattern of synaptic impairment that defines a common anatomically driven physiological phenotype. In addition, it is also known that there is an interaction between physiological and anatomical development, where anatomy develops first, and can be 'reshaped' by the ongoing maturation of physiological function (e.g., Kutsarova et al., 2017). The scientific understanding of the causative, interactive, and quantitative relationship between the two KEs is currently incomplete.

Quantitative Understanding of the Linkage

The scientific understanding of the causative and quantitative relationship between the two KEs is currently incomplete.

Response-response relationship

Although several examples are evident to demonstrate direct linkages between alterations in hippocampal anatomy and disruptions in hippocampal physiology, there is not one mechanism, anatomical insult, or signature pattern of synaptic impairment that accompanies each of these treatments. Information does not exist to develop quantitative relationships between the KEs in this KER. Papers that utilize knock-out and mutant models have not provided 'dose-response' information for anatomy-physiology relationships.

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Relationship: 748: Hippocampal Physiology, Altered leads to Cognitive Function, Decreased

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals	adjacent	High	Moderate
Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals	adjacent	Moderate	Low
Thyroid Receptor Antagonism and Subsequent Adverse Neurodevelopmental Outcomes in Mammals	adjacent	High	Moderate
AhR activation in the liver leading to Subsequent Adverse Neurodevelopmental Outcomes in Mammals	adjacent	Low	Low

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
AhR activation in the thyroid leading to Subsequent Adverse Neurodevelopmental Outcomes in Mammals	adjacent	Moderate	Moderate
Binding to VGSC during development leads to cognitive function decrease	adjacent		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	High	NCBI
mouse	Mus musculus	High	NCBI
humans	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
During brain development	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

Most work has been conducted with rodent models. Sex-specific differences in sensitivity to disruption and sex-dependent differences in behavioral performance of hippocampal tasks have been reported in both rodent models and human studies.

Key Event Relationship Description

It is a well-accepted assertion that hippocampal synaptic integrity and plasticity are essential for spatial information processing in animals and spatial and episodic memory in humans (Burgess, 2002; Martin et al., 2000; Sweatt, 2016). A large number of studies with a variety of techniques and approaches (e.g. nutritional and chemical stressors, gene knockouts) have linked hippocampal functional deficits to decreased spatial ability, context learning, and fear learning. Study of human disease states and conditions where hippocampal function is impaired (i.e., brain trauma, Alzheimer's disease, temporal lobe epilepsy, Down's Syndrome), and imaging studies of hippocampal activation during memory challenge, makes irrefutable that the hippocampus is essential for specific types of cognitive abilities. Decades of animal research has reinforced this assertion.

There are many forms of synaptic plasticity and numerous ways in which physiological function of neural circuits can be assessed. Similarly, there are many forms of learning and memory relying on different brain regions and and multiple tasks and specifics associated with these tasks that vary from laboratory to laboratory. An emerging field of computational cognitive neuroscience lies at the intersection of computational neuroscience, machine learning and neural network theory. These computational and theoretical frameworks support the participation of hippocampal synaptic transmission and plasticity in learning and memory in animals and humans (for review see: Ashby and Helie, 2012).

Evidence Supporting this KER

The weight of evidence for physiological hippocampal function and episodic memory in humans and the animal analogue, spatial and fear-based context learning, is strong. Seminal studies over the past 60 years firmly established the cellular basis of behavior with synaptic plasticity (long term potentiation and long-term depression, LTP and LTD respectively). Recent work has provided details on the local hippocampal circuitry needed for memory formation and behavioral change (Sweatt, 2016). In humans, virtual reality experiments in large-scale spatial contexts show the convergence of spatial memory performance in normal patients with fMRI of the hippocampus clearly demonstrating the essentiality of hippocampal function to spatial learning (Burgess, 2002). This assertion is consistent with a wealth of animal data on hippocampal learning and memory. In rodent models, functional impairment of the hippocampus assessed using electrophysiological techniques is correlated with deficits in spatial memory typically assessed using mazes, and memory for context often assessed in fear-based learning paradigms (O'Keefe and Nadel, 1978; Clark et al., 2000; Squire, 2004; Eichenbaum, 2000; Panja and Bramham, 2014).

Biological Plausibility

The biological plausibility of the KER is rated as strong. It is well accepted that the normal hippocampal function is critical for the acquisition and memory of context and spatially mediated tasks in rodents and humans (Sweatt, 2016).

Empirical Evidence

Empirical support for this KER is strong. The requisite of hippocampal integrity to optimal visuo-spatial context learning (i.e., episodic memory) in humans and spatial learning in rodents is well documented. In vivo recording in conscious behaving animals has demonstrated activity-dependent neural changes taking place in the hippocampus during spatial learning (Gruart and Delgado-Garcia, 2007). Impairments in hippocampal function induced by drugs, chemicals, lesions, nutritional deficiencies, mutant or knock out models that cause changes in synaptic transmission, plasticity, and hippocampal network activity, are coincident with deficits in spatial and context-based fear learning (O'Keefe and Nadel, 1978; Bannerman et al., 2014; Lynch, 2004; Verret et al., 2012). Similarly, treatments found to enhance or facilitate hippocampal synaptic transmission and plasticity are associated with improved learning and memory (Deng et al., 2010; Novkovic et al., 2015; Andrade et al., 2015; Trivino-Paredes et al., 2016). A few examples of a large literature are briefly summarized below.

It is well known that n-Methyl-d-aspartate (NMDA)-mediated glutamatergic synaptic transmission is essential for the induction of hippocampal synaptic plasticity in the form of LTP. Blockade of this form of plasticity by selective NMDA-receptors blockers impairs LTP and hippocampal tests of learning and memory (reviewed in Sweatt, 2016). Perturbation of hippocampal plasticity and impaired spatial learning have been reported in adult offspring following prenatal ethanol exposure (An and Zhang, 2015). Developmental morphine exposure caused decrease in the amplitude and slope of fEPSC sand inhibition of LTP in CA1 neurons fEPSPs that resulted in decreased maze performance (Aghighi et al., 2019). Developmental nutrition deficiency and hypoxic stress are both associated with changes in synaptic structure, altered EPSPs, and hippocampal based cognitive behaviors (Dumets et al., 2020; Zhuravin et al., 2019). Rodent models of developmental TH insufficiency are associated with, impairments in hippocampal synaptic transmission and plasticity and are coincident with deficits in learning tasks that require the hippocampus (Opazo et al., 2008; Gilbert and Sui, 2006, Gilbert, 2011, Gilbert et al., 2016).

There are also a number of mutant mouse models that have linked changes in hippocampal physiology with alteration in cognitive behaviors. The fyn mutant mouse (fyn is a tyrosine kinase pathway) displays impairments in hippocampal synaptic transmission and plasticity, as well as spatial learning deficits (Grant et al., 1992). Brain-derived neurotrophic factor (BDNF) knockout animals exhibit synaptic plasticity deficits and learning impairments (Aarse et al., 2016; Panja and Bramham, 2014). In the Jacob/Nfsm model which also exhibits pronounced alterations in BDNF-mediated signaling, hippocampal synaptic transmission and plasticity impairments were accompanied by deficits in contextual fear conditioning and novel location recognition tasks (Spilker et al., 2016). The aryl-hydrocabon (AhR) knockout was shown to decrease hippocampal mossy fibers and also impair maze performance (Powers et al., 2005).

Knockout of SALM4/Lrfn3, a synaptic adhesion molecule that modulates NMDA receptor function, increases NMDA-mediated currents and enhances contextual fear memory. In this model, control level of performance could be restored via treatment with fluoxetine, a selective serotonin reuptake inhibitor (Lie et al.,2021). Finally, a knockout of LIMK-1, a kinase associated with actin dynamics, was shown to alter hippocampal spine morphology and LTP, with subsequent changes in fear behaviors and a spatial learning task (Meng et al., 2002).

In humans, hippocampal physiology assessed using neuroimaging reveals activation of hippocampus upon engagement in spatial learning and episodic memory providing a direct linkage of these two specific KEs (Burgess, 2002). In fMRI studies of congenitally hypothyroid children, or children born to women with altered thyroid function during pregnancy, changes in hippocampal activity patterns during memory encoding and retention were observed and associated with memory impairments (Wheeler et al., 2012; 2015; Willoughby et al., 2013; 2014).

Temporal Evidence

The temporal nature of this KER is developmental (Seed et al., 2005). This has been demonstrated in multiple studies. It is well-recognized that there are critical developmental windows for disruption of the functional development of the hippocampus and the integrity of this structure is essential for later development of spatial ability, context learning, and fear learning. A wealth of studies have shown correlation between hippocampal LTP and spatial learning performance, as well as the role of glutamatergic synaptic transmission and BDNF-mediated signaling pathways in these processes (Bramham, 2007; Andero et al., 2014; Morris et al., 1986; Sweatt, 2016; Migaud et al., 1998). Although studies on reversibility are relatively rare, but a few examples of deficits in hippocampal synaptic transmission and plasticity documented in knockout mouse models are described above. In addition, in slices from BDNF knockout animals, physiological function can be rescued with recombinant BDNF (Patterson et al., 1996).

Uncertainties and Inconsistencies

There are no inconsistencies in this KER, but there are some uncertainties. It is a widely-held assertion that synaptic transmission and plasticity in the hippocampus underlie spatial learning (Martin et al., 2000; Gruart and Delgado-Garcia, 2007; Bramham, 2007). However, the causative relationship of which specific alterations in synaptic function are associated with specific cognitive deficits is difficult to ascertain given the many forms of learning and memory, and the complexity of synaptic interactions in even the simplest brain circuit.

Quantitative Understanding of the Linkage

Information does not exist to develop quantitative relationships between the KEs in this KER.

Response-response relationship

Limited dose-response information is available. Mutation and knockout mouse models are not conducive to examination of varying levels of impairment at the physiological or behavioral level. Studies have investigated dose-dependency of impairments in hippocampal electrophysiological and behavior have been reported in animals suffering from developmental TH

insufficiency (e.g., Gilbert and Sui, 2006; Gilbert, 2011; Gilbert et al., 2016).

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