Chapter 1
Neurobehavioral Comorbidities of Epilepsy: Lessons from Animal Models

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Abstract Animal models can afford useful insights into the mechanisms of neurobehavioral comorbidities of epilepsy. However, clinical relevance and value of the information that can be extracted from animal studies depend on many factors, including choice of proper models of epilepsy, choice of proper behavioral tasks, and accounting for the presence of multiple concurrent neurobehavioral disorders in the same epileptic animal. This chapter offers an overview of approaches used to examine selected neurobehavioral comorbidities in animal models of epilepsy. Assays used to study spatial and object memory, depression, anxiety, attention deficit/hyperactivity disorder, psychosis, and autism are described. First, the approaches are presented from a standpoint of single comorbidity, and mechanisms underlying respective epilepsy-associated neurobehavioral abnormalities are discussed. Further, examples are given as to how concurrent neurobehavioral perturbations may influence one another, and therefore how this may affect outcome measures and interpretation of the obtained data. It is suggested that systemic approach, rather than more commonly used isolated approach, offers more clinical-relevant and complete description of multifactorial systems that underlie neurobehavioral comorbidities of epilepsy.

Keywords Epilepsy • Behavior • Animal models • Cognition • Memory • Depression • Anxiety • Attention deficit/hyperactivity disorder • Psychosis • Autism

Do We Need Animal Models of Epilepsy Comorbidities?

Recent technological advances have contributed to a remarkable progress into understanding mechanisms of neurobehavioral disorders in epilepsy patients. Yet, clinical systems have inherent limitations which hinder both mechanistic studies

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and clinical trials. Among such limitations, to name a few, are medications which interfere with natural evolution of the disease, and in addition may themselves produce neurobehavioral side effects; difficulties with enrolling homogenous and quantitatively sound cohorts amenable to statistical analysis; psychosocial factors which can exacerbate biological aspects of the disease (e.g., stigma can further exacerbate mood disorders); unaccountable environmental factors; ethical concerns; and, particularly when it comes to clinical trials, compliance and safety considerations.

Most if not all of these limitations can be overcome, or at least substantially mitigated, through the employment of animal systems. Indeed, in experimental animals, the disease can be allowed to take its natural course without treatment interference, thus facilitating mechanistic insights; sample size is only limited by regulatory requirements (such as refinement, reduction, replacement, known as "3R" [1, 2], by the capacity of the laboratory and by the costs; the subjects are generally genetically homogenous and can be enrolled on demand by age and gender; biological aspects of the comorbidities are not contaminated by psychosocial factors (such as stigma, work environment, etc.); environmental and compliance concerns are limited or nonexistent.

Therefore, the questions are not whether animal models are needed, but how closely they reflect real-life scenarios and to what extent the information extracted in the laboratory setting is clinically relevant. With this regard, a long-standing skepticism has persisted in clinical milieu, which is particularly understandable when it comes to animal models of neuropsychiatric disorders. The liability lies with both sides. On the one hand, there is certain misconception as to the purpose of animal models, which by design is not expected to replicate a real-life system, but rather to facilitate its comprehension through simulation and visualization. On the other hand, for laboratory scientists, animal experiments frequently become a self-serving activity, when research is performed for the sake of the research, and clinical considerations are not factored into the study design and goals.

The purpose of this chapter is not to merely recite literature on neurobehavioral comorbidities of epilepsy, but to attempt narrowing the gap between clinicians and laboratory researchers through analysis of approaches used to model neuropsychiatric comorbidities of epilepsy.

Animal Assays Used to Examine Neurobehavioral Comorbidities of Epilepsy

In laboratory animals, the information cannot be obtained through self-reporting questionnaires of interviews. Hence, behavioral assays for neuropsychiatric disorders frequently have to rely on anthropomorphism; that is, the experimenter poses a question of how he or she would have behaved adequately under certain conditions and projects such apparent behavior on the animal. The expected responses are corrected to factor in the knowledge about species-specific behaviors (e.g., sociability

of rodents, their preference toward dark vs. lit areas, etc.). Any deviation from a behavior deemed adequate by the researcher is then interpreted as pathological. Admittedly, there is always a strong subjective component in interpreting an animal's response. This bias can be mitigated by subjecting an animal to more than one behavioral test to examine a disorder of interest.

When validating behavioral tests and models for studying neuropsychiatric disorders, two principles are attempted to be abided by face validity and construct validity [3]. Face validity means resemblance of animals' behavior to behavior in humans, under similar conditions (but corrected for species-specifics). For example, avoiding the engagement with other animals of the same species would suggest good face validity for animal models of autism. Construct validity means resemblance of known underlying mechanisms. For example, the dysfunction of serotonergic transmission suggests good construct validity of a system for reproducing major depressive disorder. Better models would offer both good face validity and construct validity, although this is not always the case. For example, spontaneously hypertensive rats (SHR) present with symptoms of attention deficit and hyperimpulsivity and are deemed having good face validity for modeling attention deficit/hyperactivity disorder (ADHD) [4, 5]; construct validity of the system however is poor, as hypertension, which is inherent to the strain, is not a symptom of ADHD. When it comes to preclinical trials, predictive validity also comes in play, as the one reflecting the ability of a medication tested in an animal model to correctly predict the efficacy of this drug in a homologous human disorder [3]. Here, reverse translation is often applied. For example, if in a given animal model of depression, clinically available selective serotonin reuptake inhibitors (SSRI), fluoxetine and citalopram, effectively improve depressive behavior, then it is assumed that the model has good predictive validity, as novel investigational drugs effective in this model would also have therapeutic effects in patients.

Here, due to space restrictions, only most commonly used behavioral assays are discussed. Furthermore, the discussion is limited to rats and mice as most commonly used species in epilepsy and behavioral research. Finally, as mechanisms which drive various behaviors are complex and multifaceted, only select mechanisms are addressed.

Spatial Recognition and Memory

Morris water maze (MWM) is a common way to test animal's spatial recognition and memory [6–8]. The test relies on the fact that rodents are good, but not keen swimmers, and thus would avoid swimming if possible. The apparatus is a large cylindrical tank filled with water with a submerged escape platform placed in one of the virtual quadrants. When the animal is first placed in the tank, it swims aimlessly, eventually stumbles upon the platform and climbs on it (Fig. 1.1a; if exploratory swimming exceeds the set duration, the animal is manually guided to the platform). During the learning phase, as the task is repeated several times a day, normal

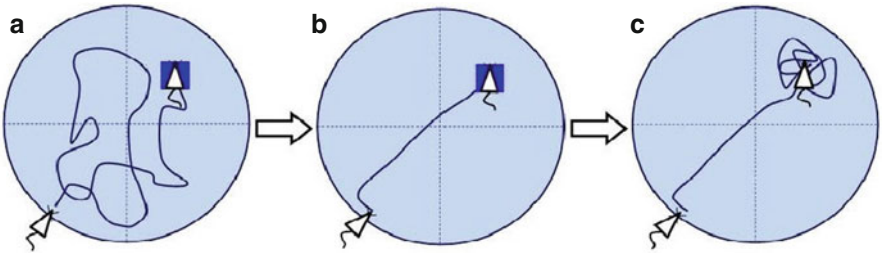

Fig. 1.1 Morris water maze (MWM) test for spatial learning and memory. Schematic rendering of spatial learning and memory in the MWM. Animal's movements around the tank are outlined by trace lines. (**a**, **b**) Spatial learning. (**a**) During the initial trials, upon placement in the tank, the animal swims around aimlessly, finds the platform (represented by the *blue square*) by accident, and climbs on it. (**b**) As the trials are repeated for several days, the animal learns the location of the platform and swims to it directly. (**c**) Spatial memory. After training, the platform is removed. Nevertheless, the animal spends more time swimming in the quadrant where the platform used to be, than in the other three quadrants of the tank

animals learn the placement of the platform, and the latency to the escape gradually declines. The training successfully culminates, when, after placing in the water, the animal swims directly to the platform and climbs on it, rather than explores various areas of the tank (Fig. 1.1b). The number of trials needed for the animal to learn the placement of the platform serves as a measure of spatial learning. During the retrieval phase, after initial training, the platform is removed. Normal animals spend more time swimming in the quadrant where the platforms used to be than in the other three quadrants of the tank (Fig. 1.1c). Total time spent in the relevant quadrant serves as a measure of spatial memory. Therefore, animals for which it takes longer to learn the platform location during the learning phase, and those which divide time equally among the four quadrants of the tank during the retrieval phase, are interpreted to have spatial cognitive impairments.

Among various mechanisms determining spatial cognition and memory, one can be singled out as particularly important. The processes of spatial recognition and memory are driven by a subset of pyramidal cells in the hippocampus called, due to their function, place cells [9, 10]. Single place cell fires each time the animal enters a particular location in the environment (known as place field). In the confined environment (such as MWM), the activation of each place cell is typically associated with a single place field. As the animal moves around the area, different place cells are activated. Collectively, place cells act as a representation of a specific location in space, thus forming a cognitive map [11]. Cognitive maps can be built by recording from single place cells as the animal moves around the enclosure, typically a cylinder. On the first training day, fasting animal is placed inside the cylinder, where food pellets are scattered on the floor so as to ensure that the animal visits all parts of the cylinder. On subsequent days, single food pellets are dropped consecutively and randomly at various locations, so that the animal travels along various paths. While the animal moves around the enclosure, the activity of single place cell is recorded. The resulting firing field reflects the activity of the recorded place

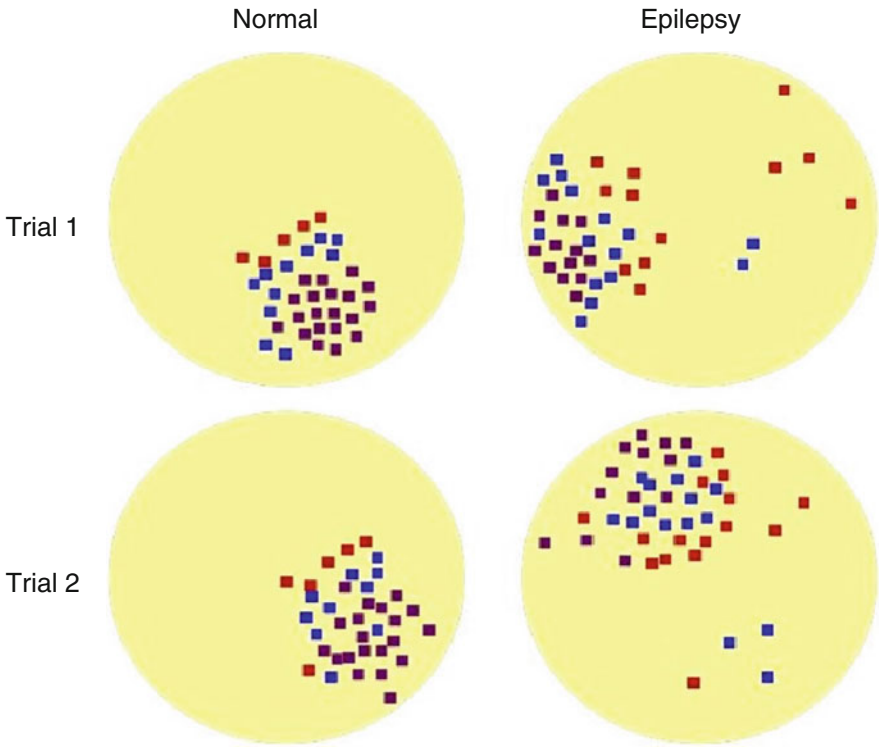

Fig. 1.2 Example of the stability of place cell firing patterns in a normal rat, and a rat with experimental MTLE. Schematic compilation of real-life place cell maps [18, 20–22], showing firing rate of a single place cell, while the animal is moving around the cylindrical enclosure, in two consecutive trials. Pixels reflect firing rates coded in the sequence, *yellow* (no firing)–*red*–*blue*–*purple* (highest firing rate) colors. In normal rats, the firing pattern is consistent between the trials 1 and 2. In animals with MTLE, the fields vary from one trial to another

cells with reference to different parts of the cylinder. In MWM, learning of the location of the platform and its retrieval is associated with firing of specific place cells, which "guide" the animal to the target area (these processes are known as place cell preplay and replay, applied to learning and memory, respectively) [12, 13] (Fig. 1.2).

Understandably, any dysfunction or loss of place cells, such as it occurs under conditions of mesial temporal lobe epilepsy (MTLE), is expected, and may in turn translate into deficits of spatial learning and memory.

Indeed, impaired performance in MWM and concurrent dysfunction of place cells has been reported in several experimental models of epilepsy. Status epilepticus (SE) induced in rodents by pilocarpine (or LiCl and pilocarpine combination) produces a variety of perturbations resembling human MTLE, such as spontaneous recurrent seizures developing after a brief "silent" period; extensive neurodegeneration and gliosis in the hippocampus; formation of aberrant excitatory hippocampal connections, etc. [14–16]. Interictally, epileptic animals present with profound

deficits in MWM performance, including larger number of trials needed to learn the location of the platform during training, as well as poor recall of platform location during retrieval phase [17–19]. These behavioral aberrations correlate strongly with the dysfunction of place cells. Particularly, the stability of place cells (i.e., consistency of firing during different trials associated with the same location in the cylinder) is diminished, as well as their precision with the reference of certain location within the area [18, 19]. Figure 1.2 shows schematic rendering of typical firing patterns of single place cells in normal animals and those with chronic epilepsy.

Furthermore, even potentially epileptogenic insults, which produce no explicit epilepsy (i.e., seizures or neurodegeneration), result in long-lasting memory deficits and dysfunction of place cells. As such, these impairments have been observed in adult rats which underwent a series of primary generalized flurothyl-induced seizures [20, 21] or hyperthermia-induced seizures [22] during neonatal age; neither of these protocols produces spontaneous recurrent seizures, nor is it accompanied by explicit histopathology, but at the same time decreases seizure threshold, thus creating increased susceptibility to a secondary epileptogenic hit. The latter findings are particularly important, as they emphasize that impairments of spatial memory are not a direct consequence or an artifact of recurrent seizures, but rather have specific underlying mechanisms, which are triggered by the same insult as epilepsy, but progress on their own volition, independently of epileptogenesis proper.

Nonspatial (Object) Learning and Memory

Object memory is examined in the novel object recognition test, which is based on visual discriminative ability coupled with the natural curiosity, typical for rodents [23].

During the learning phase, the animal is placed in the confined environment, where it is presented for the exploration with two objects of distinct shapes (e.g., cube and pyramid). Normally, animals spend about similar time exploring each of the objects. After a period of exploration, the animal is removed, and after some time (generally 6–24 h), it is returned to the task area, where one of the objects is now replaced with a different one (e.g., the cube is replaced with a cylinder). During this phase, normal animals spend more time exploring the new object as compared with an already familiar one, while animals with impaired object memory treat both objects as novel and thus equally divide their time between the two. The proportion of time spent exploring novel versus familiar object is used to measure object memory.

Short-term object memory is primarily driven by the activity of rhinal and perirhinal cortices [24, 25], while long-term memory involves hippocampus as well [26], so that both short-term and long-term object memory can be affected in MTLE.

Impaired object memory has been reported in rats with pilocarpine SE-induced chronic epilepsy [27], although this has not been universally accepted [17]. Similar to spatial memory, potentially epileptogenic factors, such as cortical dysplasia

induced by in utero irradiation [28], or a single primary generalized seizure induced by pentylenetetrazole, resulted [29] in the long-lasting impairments in object memory, even in the absence of recurrent seizure activity. Importantly, epilepsy-associated deficits in spatial and object memory are not redundant, as model-specific differences have been reported; thus, while single seizure episode resulted in the prolonged object memory disruption, while spatial memory was not compromised [30], animals with post-SE MTLE have been reported to present with spatial memory impairments, while object memory was spared [17].

Depression

Most commonly examined symptoms of depression are hopelessness/despair and anhedonia (i.e., inability to experience pleasure). Examination of the state of despair/hopelessness is performed using variations of the forced swimming test (FST) [31–33]. The test is designed to gauge animals' ability (or inability) to effectively cope with an inescapable stressful situation. The latter is created by placing the animal in a cylindrical tank filled with water, with no possibility to escape (e.g., platform, rope, etc.). Animals display several behavioral patterns, with two types prevailing. Active escaping behavior is characterized by climbing on, or swimming along the walls, or diving, and it is interpreted as effective coping. This behavior intermits with periods of immobility, where the animal passively floats, and movements are limited to maintaining the head above water so as to avoid drowning; when immobile, animals are thought to give up in their attempts to escape (Fig. 1.3). While both normal animals and those with experimentally induced depression display both types of behavior, in models of depression, such as depression-prone inbred strains of rats [33], mice with targeted mutations relevant to depression (e.g., overexpression of serotonin [5-HT] type 1A autoreceptors [34]), olfactory deprivation achieved by surgical removal of olfactory bulbs [35], chronic mild stress (e.g., repeated immobilization) [36], or Gram-negative bacterial infection mimicked by lipopolysaccharide (LPS, a state known as LPS sickness) [37], the immobility time significantly increases, whereby animals may spend most of the time immobile, rather than attempting to escape. Such exacerbated immobility is interpreted as a state of hopelessness/despair, and the state can be quantified by calculating the immobility to active swimming ratio. Indeed, the duration of immobility is effectively reduced by chronic treatment with antidepressants (such as SSRI and MAO inhibitors) [31, 32].

In mice, tail suspension test (TST) is frequently performed in lieu of FST [39]. In the TST, an inescapable stressful situation is created by suspending the mouse by the tail and quantifying active behavior (i.e., struggling attempts to free up) and immobility. These behaviors parallel respective patterns in the FST and are also amenable to standard antidepressant medications.

Another core symptom of depression, anhedonia, is examined using taste preference test. The test relies on innate affinity of rodents toward sweets [40]. When

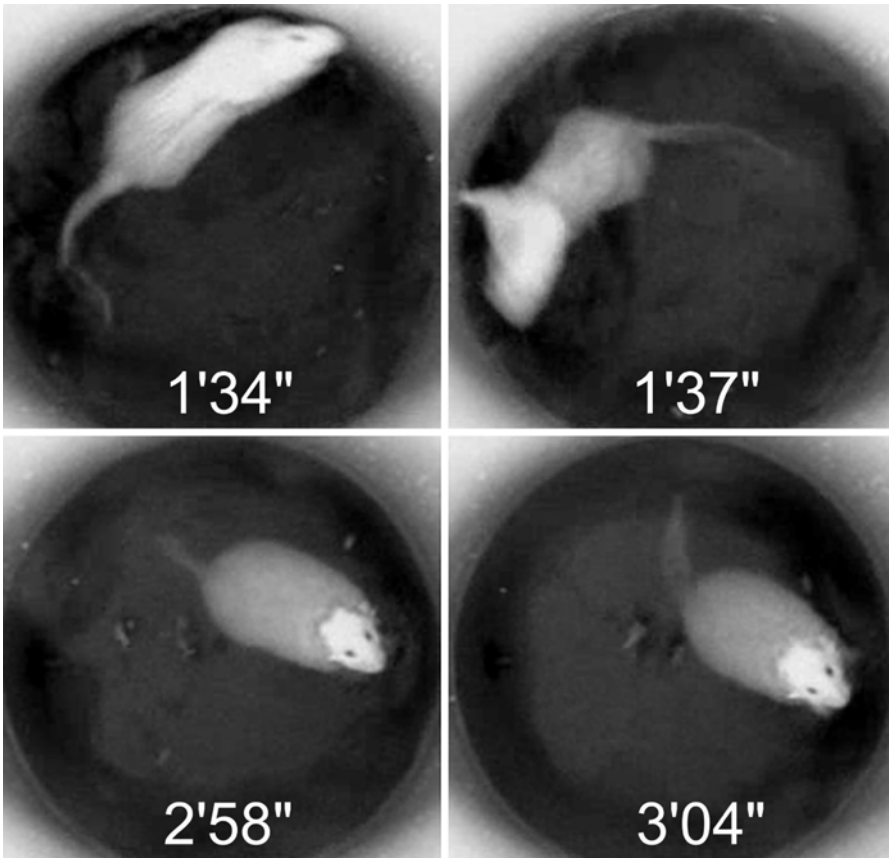

Fig. 1.3 Behavioral patterns in the forced swimming test (FST) for depression. Sample snapshots taken from pre-recorded video during FST. Time after the start of the test is indicated on each image. Examples of active swimming, which reflects active escape strategies, are presented at 1 min 34 s and 1 min 37 s. Note the change in the rat's position in the tank, which occurred during the 3-s period, and the fuzziness of images due to the animal's movement. Examples of immobility when animals move only enough to avoid drowning are presented at 2 min 58 s and 3 min 04 s. Note that the animal's position in the tank did not change during 6 s of recording and that the body is positioned vertically in the water [38] (Reprinted from Mazarati et al. [38], with permission from Elsevier)

presented with the choice of two bottles, one filled with water and another with sweet solution (e.g., low percent saccharin or sucrose), normal animals preferentially consume the latter. "Anhedonic" animals show no drink preference, but consume statistically equal volumes of water and saccharin. The presence of anhedonic state is thus measured by the consumed saccharin (sucrose) to water ratio.

Among the factors regulating behavior in depression tests, ascending serotonergic pathways (i.e., those emanating from raphe nuclei, and projecting to prefrontal cortex [PFC] and hippocampus) play a critical role [41, 42]. In turn, the release of serotonin from raphe into target areas has complex regulatory

mechanism, but two are most notable: short feedback autoinhibitory loop involving 5-HT1A raphe autoreceptors [43–45] and descending glutamatergic (i.e., excitatory pathway) from PFC into the raphe [46]. Hence, such perturbations, as the upregulation of 5-HT1A autoreceptors or a diminished excitatory drive from PFC in the raphe, will translate into the diminished 5-HT output, and consequently, into depression.

Another important mechanism is the dysregulation of hypothalamo-pituitary-adrenocortical (HPA) axis. The HPA axis dysregulation occurs because of the compromised negative feedback mechanism, whereby cortisol (or corticosterone in rodents) released from adrenal cortex fails to suppress corticotrophin-releasing hormone (CRH) release from hypothalamus and/or adrenocorticotropic hormone (ACTH) release from the anterior pituitary. As a result, the response of the HPA axis to stressors becomes unabated and maladaptive [47, 48]. This has many central and peripheral consequences. One of such consequences, particularly relevant to depression, is the upregulation of raphe 5-HT1A autoreceptors [48–50], which, as discussed earlier, would lead in turn to the increased autoinhibition of serotonin release and its insufficient delivery into forebrain structures. The dysregulation of the HPA axis can be revealed using either dexamethasone (DEX) suppression test or the combined DEX/CRH test (both are also used in patients). The latter consists of measuring corticosterone plasma levels in response to the administration of DEX (which normally suppresses plasma corticosterone), followed by the administration of CRH (which normally increases plasma corticosterone). Blunted response to DEX and exacerbated response to CRH represent objective measures of the HPA axis hyperactivity [51].

Increased immobility time in the FST in rats and mice, or in TST in mice, as well as anhedonia have been reported in several models of MTLE [52–54], as well as models of absence epilepsy [55, 56]. It has been suggested that epilepsy-associated depression develops as a result of cascade of events, starting with brain inflammation. Brain inflammation, and particularly, the increased signaling of a cytokine interleukin-1β (IL-1β) is triggered by a precipitating insult (e.g., status epilepticus or traumatic brain injury), and may be sustained by recurrent seizures [57, 58]. IL-1β had been reported to facilitate or even precipitate seizure activity through the phosphorylation of NR2B subunit of the NMDA receptor [59]. At the same time, chronic inflammation has been implicated in mechanisms of depressive disorders [60] (one remarkable observation is a high prevalence of depression among patients with rheumatoid arthritis [61]). In animal systems, chronic inflammation, induced by the administration of LPS (an endotoxin of Gram-negative bacteria), produced a spectrum of depressive impairments, including despair/hopelessness and anhedonia in respective tests [37, 62]. One of the consequences of the activation of IL-1β signaling in animals with chronic epilepsy is the dysregulation of the HPA axis [38, 53]. The resulting sustained high levels of circulating corticosterone in turn upregulate 5-HT1A autoreceptors in raphe nuclei [63]. Upon the 5-HT1A autoreceptor upregulation, the resulting increased autoinhibition of serotonin release culminates in the development of depressive behavioral abnormalities. Indeed, treatment of epileptic/depressed animals with an IL-1 receptor blocker (IL-1ra, also known as anakinra) disrupted both neuroendocrine and behavioral symptoms of depression [38].

It should be noted that the presence of symptoms of depression in epileptic animals has not been universally accepted. In fact, several studies have found that animals with post-SE chronic epilepsy display decreased, rather than increased, immobility in the FST and TST [64, 65]. Such findings can be interpreted in one of the following ways: either seizures produce a true antidepressant effect or other events interfere with the swimming ability of epileptic rats, thus rendering commonly used behavioral tests inappropriate for studying depression. One such scenario is discussed further below under Multiple Concurrent Comorbidities.

Anxiety

General anxiety is most commonly examined using elevated plus maze test (EPMT) or its variations [66, 67]. The apparatus is composed of two perpendicularly crossing walking beams (arms): one is open and exposed to the light, and the other is closed (i.e., covered, so as to create the dark tunnel, Fig. 1.4). The apparatus is elevated over the ground, so as to create an insecure environment in the open arms. The animal is able to travel along each of the arms freely. Behavioral pattern in the EPMT is a result of the balance between animal's curiosity (i.e., exploration of all arms) and insecurity (when traveling along the elevated open arms). In rodents with general anxiety, time spent in closed arms is significantly longer than in normal animals. Anxiolytic medications increase the presence in open arms.

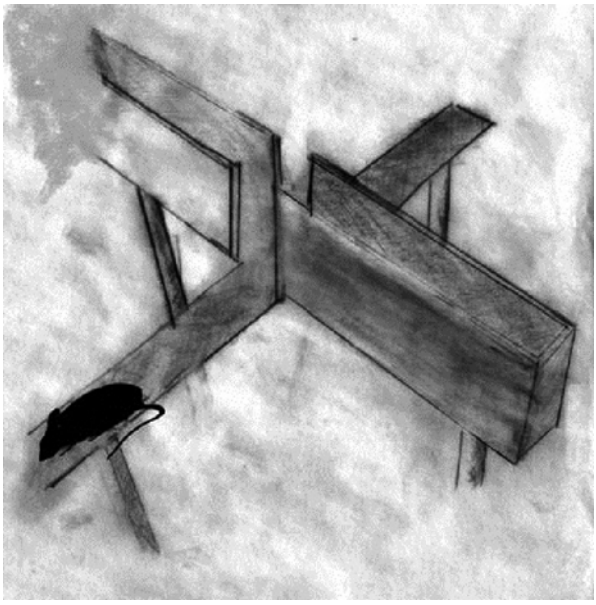

Fig. 1.4 Elevated plus maze test for general anxiety. Schematic rendering of the apparatus, with the test animals located in one of the open arms. For the description of the apparatus and the procedure, see text

The presence of general anxiety in epileptic animals has been controversial. Increased anxiety has been reported in several models of MTLE [65, 68] and absence epilepsy [69]. However, other studies reported the opposite effect of recurrent seizures, whereby epileptic animals exhibited decreased levels of anxiety in the EPMT [17, 70]. Such conflicting findings resemble those reported for depression, with two similar interpretations: either recurrent seizures produce anxiolytic effect or, for some reasons, tests for general anxiety, which work well in normal animals, become inappropriate in animals with chronic epilepsy. The latter possibility is also discussed later under Multiple Concurrent Comorbidities.

Social anxiety can be examined using a three-chamber sociability test, which is also commonly employed to study autism-like behavior (see below). Discerning between social anxiety as a stand-alone condition versus the one associated with autism may be complicated.

Attention Deficit Hyperactivity Disorder (ADHD)

The examination of ADHD relies on complex operant behavioral tasks with positive reinforcement (generally food pellets presented after a period of fasting). Commonly used variations include five-choice serial reaction time task (5-CSRT) [71] and lateralized reaction time task (LRTT) [4], but the principle is the same. In the LRTT, the operant chamber is equipped with five apertures on one side (Fig. 1.5). Each of the apertures can be lit up by a beam of light, which is delivered in an organized sequence. On the opposite side is the photocell-equipped food-pellet feeder. The animal is trained such that poking the nose in the aperture, which is about to light

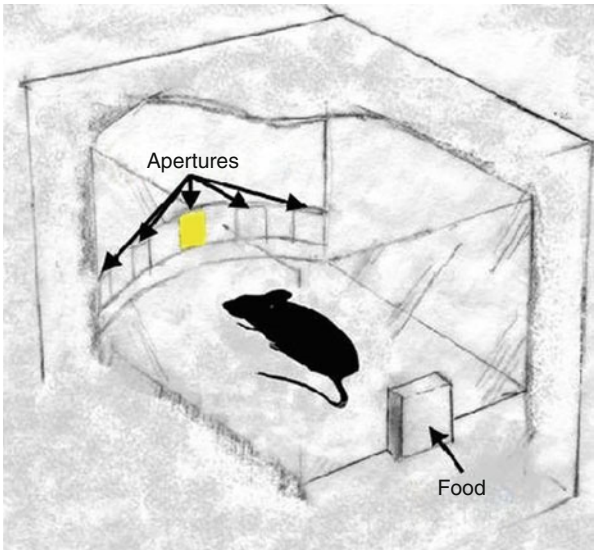

Fig. 1.5 Lateralized reaction time task (LRTT) for attention deficit/hyperactivity disorder. The chamber is soundproof. Five nose-poke apertures are in front of the rat; one of them is lit up. Photocell-equipped food pellet feeder is behind the animal. Poking the nose in the aperture which is about to light up is accompanied by the automated pellet delivery. During the test, the animal moves between the apertures and the feeder

up, is followed by the automated delivery of the food pellet. If the animal pokes the correct aperture at the correct time (e.g., 0.1, 0.5, 1 s, etc., before the light comes up), the food pellet is delivered from the feeder. Poking incorrect aperture reflects lack of attention; poking correct aperture prematurely reflects hyperimpulsivity. In neither of these cases, food reward is provided. Therefore, the numbers of incorrect and premature responses represent measurements of attention deficit and hyperimpulsivity, respectively.

Two neurotransmitter systems have been primarily implicated in the mechanisms of ADHD. Ascending dopaminergic projections from ventral tegmental area (VTA) into PFC (i.e., mesocortical pathway) and into nucleus accumbens (i.e., mesolimbic pathway), as well as noradrenergic projection from locus coeruleus (LC) into PFC play important roles in reward, impulsivity, and attention, and are compromised in ADHD [72–74]. Indeed, medications approved for the treatment of ADHD are dopamine-reuptake inhibitor methylphenidate and selective norepinephrine-reuptake inhibitor atomoxetine [75].

Animals with SE-induced MTLE present with both attention deficit and hyperimpulsivity. Real-time measurements of noradrenergic transmission by means of fast-scan cyclic voltammetry (FSCV) revealed that ADHD-like impairments develop due to the diminished norepinephrine output from LC into PFC. Furthermore, similar to epilepsy-associated depression, the observed noradrenergic deficit results from the increased autoinhibition of neurotransmitter release [76]. In case of norepinephrine, the upregulation of α2A adreno-autoreceptors in LC is responsible for the suppressed transmitter release I in the LC–PFC pathway.

Early-life primary generalized seizures produced in immature rodents by flurothyl result later in life in long-lasting ADHD-like abnormalities, even in the absence of explicit ictal events. In these animals, behavioral perturbations parallel the increased thickness of PFC [77]. Furthermore, direct administration of a GABA-A receptor blocker bicuculline into the PFC of immature rats results in transient interictal spiking in this area and subsequent long-lasting ADHD-like behavioral abnormalities [78]. These findings outline a different scenario of epilepsy-associated ADHD, which develops due to primary sustained dysfunction of PFC, rather than due to compromised LC–prefrontal cortex noradrenergic transmission. Similar to memory impairments, ADHD may represent either a true comorbidity of epilepsy (i.e., the two conditions coexist) or a consequence of early-life seizure event, even when seizures are no longer present.

Psychosis

Two tests are commonly used in rodents: locomotor activity in response to psychostimulants and the acoustic startle response (ASR; the latter is also used in patients in the diagnosis of schizophrenia).

Psychostimulant-induced locomotor response reflects the hypersensitivity of dopaminergic neurotransmission, which is a recognized mechanism of schizophrenia [79]. In turn, several variants are employed, such as amphetamine-induced

hyperactivity in the "open field" and apomorhine-induced rearing and climbing [80]. In the amphetamine test, locomotor activity is quantified by counting the number of virtual squares crossed by the animal in the confined square area (known as "open field") over a set period of time, before versus after amphetamine administration. For the apomorhine test, the animal is placed in a small cylinder, with walls constructed as grid amenable to climbing; the climbing activity is scored before and after apomorhine injection. Animals with psychosis-like impairments display significantly steeper increase in locomotion upon amphetamine administration, and more climbing on the walls of the cylinder upon apomorhine administration than healthy controls.

The rationale for ASR in rodents is similar to that in patients with schizophrenia [81, 82]. It is based on the ability of the nervous system to adapt to a stronger sensory stimulus when a preceding signal is given as a warning. The test involves prepulse inhibition (PPI) protocol, and is performed in a startle chamber which detects whole-body mechanical reaction in response to an acoustic startle stimulus, which exceeds the background noise [83]. Acoustic prepulse stimulus is followed by a pulse stimulus after a set period, and the movement induced by the pulse is measured. In normal animals, the response to the pulse is inhibited when prepulse is presented; animals with psychosis-like impairments, in which sensory adaptation is compromised, show no inhibition of startle response in the prepulse–pulse sequence.

ASR has been extensively studied in the kindling model of MTLE. Repeated, initially subconvulsive electrical stimulations of limbic structures (e.g., hippocampus, amygdala, perirhinal cortex) lead to the occurrence and progressive development of complex partial seizures (hence, the kindling phenomenon) [84–87]. Kindled animals do not typically develop spontaneous seizures or profound histopathology associated with MTLE (although both may develop after very high number of stimulations). However, kindled animals respond with secondary generalized complex partial seizures in response to the stimulus, which is inconsequential in normal rats long after the kindling procedure has been completed (therefore, kindling can be described as a chronic epileptic state without spontaneous seizures). Kindled animals show exacerbated ASR, as well as exacerbated psychostimulant-induced locomotion [88–90]. Impaired ASR has been reported in other models of MTLE (e.g., SE induced by pilocarpine or kainic acid [91, 92]), as well as in a genetic model of absence epilepsy in rats (in Genetic Absence Epilepsy Rats from Strasbourg, GAERS) [93].

Autism

In rodents, most commonly examined symptoms of autism are impairments in sociability and repetitive behavior.

Animals' ability for social engagement is most commonly examined using the three-chamber sociability test [94–96]. The test is performed in a box divided into three connecting chambers. During the first (sociability) phase, one of the terminal chambers contains an unfamiliar rodent of the same species (conspecific), and the

opposite terminal chamber – an unanimated object (Fig. 1.6). Both conspecific and the object are placed inside cylindrical wired cages, so as to isolate them from the rest of the space. The test animal is placed inside the box and is allowed to explore it freely. Animal's behavior is a result of the balance between general curiosity (manifested as exploration of both the object and the conspecific) and sociability (manifested as preferential engagement with conspecific over the object). Indeed, normal animals spend more time exploring/engaging with the conspecific, than with the object. In models of autism, however, animals show no conspecific versus object preference, and divide their time between the two equally. During the social novelty phase, which immediately follows the examination of sociability, the object is replaced with another novel conspecific, while the conspecific from the first phase remains in place, and the test is repeated. Now, normal animals spend more time engaging with the novel conspecific than with the already familiar one, while animals with autism-like impairments once again show no novel versus familiar conspecific preference.

In immature animals (between the time of birth and weaning), sociability is examined by assessing their interaction with the separated dame [96]. Pups, when separated from the dame, emit ultrasonic calls of certain modalities. In animal models of autism, the modality of ultrasonic vocalizations is altered in a specific fashion, presumably reflecting atypical vocalizations seen in autistic infants (e.g., the pups emit fewer harmonic, and more complex and short syllables [97, 98].

Self-directed repetitive behavior is analyzed by counting the duration of grooming during a set period (typically 10 min) [99]. In normal animals, grooming is episodic, while animals with autism-like abnormalities spend excessively long periods grooming, which is interpreted as the presence of self-directed repetitive behavior.

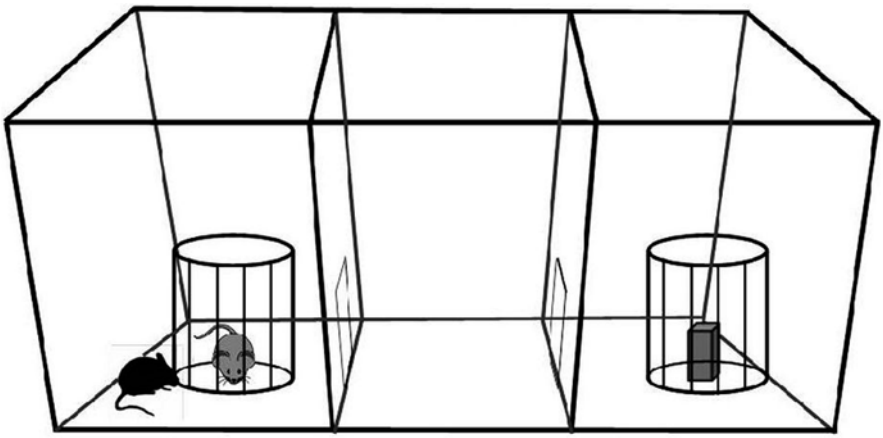

Fig. 1.6 Three-chamber sociability test for autism-like behavior. Shown is the sociability phase. Test mouse is shown in *black* in the left compartment. Conspecific mouse (in *gray*) is in the enclosure in the same compartment, and an unanimated object is inside the similar enclosure in the right compartment. Time spent exploring conspecific versus the unanimated object is counted. For the social novelty phase, the object is replaced with another conspecific, and the time spent exploring the familiar mouse (*left*) versus the novel one is calculated

Modeling autism in the laboratory reflects accepted views on the causes of the disease. Several inbred strains exist, which are characterized by behavioral and histopathological correlates of autism. BTBR mice are by far most commonly used [97, 99, 100]. These mice present with such core symptoms of autism as impaired sociability, repetitive and restricted behaviors, as well as impaired ultrasonic calls during neonatal age. Histopathologically, BTBR mice are characterized by missing corpus callosum, which reflects impaired long-range connectivity, typical of autism patients. Therefore, BTBR mice have generally good face and construct validity for modeling the disease. Other inbred strains with similar autism-like perturbations are represented by BALB/cByJ and C58/J mice [101]. Alongside inbred animals, multiple types of animals with targeted mutations are available, for example, Fmr1 knockout mice are used as a model of Fragile X syndrome [102]; Shank1 knockout mice [103] and oxytocin receptor knockout mice [104] reflect the implicated role of SHANK gene mutations and of oxytocin deficiency, respectively, in the mechanisms of autism. Finally, several models reflect the role of environmental factors in autism. The offspring of rats and mice which were treated with valproic acid during pregnancy presents with autism-like behavioral impairments [105]. Maternal immune activation (MIA) mimicked in pregnant rodents by either polyinosinic–polycytidylic acid (Poly I:C, a viral mimic) or LPS results in the offspring with impaired sociability, restricted and repetitive behaviors, and dysfunctional ultrasonic calls in neonates [98, 106].

Following the path of modeling autism proper, models of comorbidity between autism and epilepsy employ both genetic and environmental approaches. Dravet syndrome, which is caused by haploinsufficiency of the SCN1A gene encoding voltage-gated sodium channel NaV1.1, is characterized by recurrent intractable seizures and autism-like spectrum disorder [107, 108]. SCN1A (+/−) mice represent a model of Dravet syndrome with both good face validity (i.e., recurrent seizures and autism-like impairments) and construct validity [109]. Mice lacking adenomatous polyposis coli protein (APC) [110], as well as mice with triple repeat expansion of Aristaless-related homeobox (ARX) [111], present with infantile spasms during neonatal age and develop autism-like behavioral impairments later in life.

With regard to MIA (see above), the offspring of mice treated with either Poly I:C or LPS during pregnancy does not present with spontaneous seizures; however, these animals show increased propensity to MTLE upon the introduction of a second, otherwise inconsequential, postnatal hit to the hippocampus [112]. Signaling pathways involved in the development of autism alone versus autism+epilepsy prone phenotypes in the Poly I:C-induced MIA offspring have been identified: among many components of innate immunity induced by viral infection, an inflammatory cytokine interleukin-6 appears to be solely responsible for autism without increased propensity to epilepsy, whereas concurrent activation of interleukin-6 [113] and IL-1β is necessary and sufficient for producing autism+epilepsy prone phenotype [112].

Neonatal rats treated with the combination of LPS (to mimic Gram-negative infection), doxorubicin (an antineoplastic agent to produce diffuse brain damage), and p-chlorophenylalanine (a selective serotonin neurotoxin, thus used to mimic

serotonin deficiency observed in patients with infantile spasms) produce infantile spasms, followed by severe cognitive and sociability deficits later in life [114]. This model therefore is deemed to reflect a combination of environmental influences (i.e., infection, nonspecific. and transmitter-specific neurotoxicity) as risk factors for the development of infantile spasms and autism.

Prerequisite Fitness Tests

Even before proceeding with the examination of comorbid disorders, it is important to establish that the animal's basic physiological functions and physical fitness are intact. Both excitotoxic neurodegeneration and recurrent seizures (even if infrequent and subtle) may affect the animal's ability to swim, maintain balance, consume food and fluids, taste, smell, see, etc. Therefore, the inclusion of basic fitness tests relevant to the employed behavioral assays should be a prerequisite for all such studies. Examples include Rotarod test to assess coordination and balance, swimming task with visible platform to assess appropriateness of MWM and FST, visual cliff test to assess visual perception, and quinine taste aversion test to assess anhedonia. Animals which fail in the basic tasks cannot be enrolled in behavioral studies of respective comorbidities. However, such prerequisite tests are not always performed, and thus the experiments proper may yield either false-negative or false-positive results.

Multiple Concurrent Comorbidities

Seventy years ago, Arturo Rosenblueth and Norbert Wiener described two types of scientific models – formal and material models [115]. Formal model approach, which is reductionist by nature, is most commonly employed for examining neurobehavioral comorbidities of epilepsy. In practical terms, it means that the experimental design focuses on one particular neurobehavioral disorder and its association with epilepsy, but either deliberately dismisses other variables or, when observing several concurrent disorders, does not regard them as dependent on, and connected to, one another. By virtue of being reductionist, such approach is far removed from real-life scenarios. As a result, clinical relevance of experimental findings, their proper interpretation, and applicability for preclinical trials, all become severely limited. Indeed, epilepsy patients often present with more than one neurobehavioral disorder (e.g., cognitive impairments frequently coexist with mood disorders, anxiety – with depression, etc.) [116–118]. Furthermore, epilepsy comorbidities may have complex relationships not only with seizures, but also among themselves, and thus are likely to influence each other's course and clinical manifestations.

On the upside, merely because an experimental study explores a specific comorbidity, it does not mean that this is the only neurobehavioral disorder that the animal has. On the contrary, as it has been discussed earlier, perturbations in mood, cognition,

attention, social interaction, all have been reported within the framework of a single epilepsy model. The problem therefore is not a system-limitation proper, but the fact that typical experimental design simply ignores multiple neurobehavioral impairments in favor of a single disorder, which is the primary focus of the project.

Not only such simplification overlooks the complexity of real life, but it also can lead to faulty interpretations. Indeed, similar to clinical situations, in laboratory systems, coexisting neurobehavioral comorbidities may influence one another, and therefore may skew outcome measures (e.g., the presence of anxiety will likely affect the way epileptic animal performs in cognitive tasks, and the presence of depressive disorder – performance in attention tasks; Fig. 1.7).

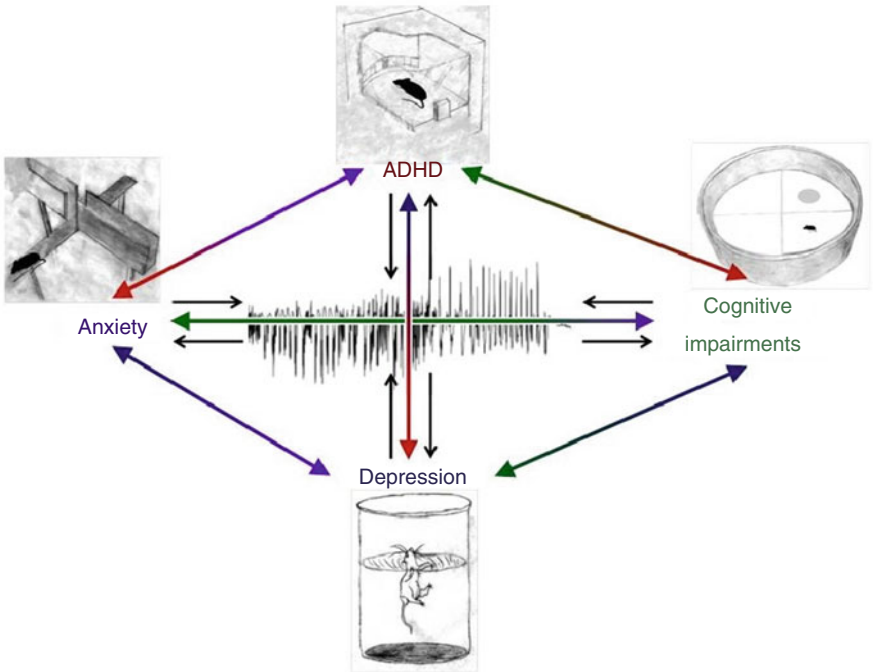

Fig. 1.7 Multiple interactions between seizures and neurobehavioral disorders in animal models of epilepsy comorbidities. Epilepsy proper (including both recurrent seizures and interictal events, such as interictal spikes) affects animal's behaviors and hence the performance in respective tests (examples given are lateralized reaction time task for ADHD, Morris water maze for spatial cognitive deficits, the forced swimming test for depression, and the elevated plus maze test for general anxiety). Furthermore, seizure–behavior interaction is often bidirectional (e.g., depression-associated suppression of serotonergic transmission or ADHD-associated compromised noradrenergic transmission may further exacerbate epilepsy). In addition, concurrent neurobehavioral disorders in animals with epilepsy interact with each other and thus may influence outcome measures. For example, the presence of depressive impairments may affect animal's performance in cognitive and memory tasks; the presence of ADHD may affect animal's ability to perform adequately in tests for depression, anxiety, etc. Such interactions emphasize the complexity of experimental animal systems and call for a systemic, rather than isolated, approach for studying neurobehavioral comorbidities of epilepsy in the laboratory

At the same time, when and if the interaction between different comorbidities is taken into account, animal studies may yield useful insights in human condition.

A case in point is comorbidity between depression and ADHD in animals with experimentally induced MTLE. Careful analysis of swimming behavior in epileptic rats during the FST has revealed complex behavioral patterns. While a majority of animals (approximately 2/3 in various experiments) show increased immobility time in the FST, thus pointing toward the presence of depressive disorder, a subset of epileptic rats exhibits increased active swimming, which was however different from the normal adaptive swimming behavior [76]. Unlike in normal rats, in the hyperactive epileptic animals, active swimming was nonadaptive: animals did not attempt to escape the tank, but rather trod water in the middle, without attempts to escape. On the surface, by merely looking at the passive-to-active swimming ratios, these animals could be categorized as the ones with reduced depressive behavior, with the conclusion that epilepsy may produce "antidepressant" effects [64, 65]. It turned out, however, that these animals, when examined in the LRTT, showed signs of hyperimpulsivity, that is, presented with symptoms of ADHD. Furthermore, looking at the biological substrate of passive versus nonadaptive swimming behaviors, it occurred that "depressed" animals displayed suppressed serotonergic tone in the raphe–PFC pathway, while "nonadaptive swimmers"/hyperimpulsive animals showed selective suppression of noradrenergic transmission in the LC–PFC ascending projection [76]. Therefore, the nonadaptive active swimming behavior observed in the FST more likely represented a manifestation of ADHD, rather than an "antidepressant" effect of seizures. This observation is also important, considering a well-known comorbidity between ADHD and depression, and the fact that in ADHD/depression patients, diagnosis of ADHD is often complicated as depression may mask symptoms of ADHD [119, 120]. Indeed, a small subpopulation of animals with chronic epilepsy (around 10–15 %), which acted only as depressed (i.e., having relevant impairments in the FST, but no hyperimpulsivity in the LRTT), was found to have compromised both raphe–PFC serotonergic transmission and LC–PFC noradrenergic transmission [76], thus suggesting that they may have ADHD alongside depression; only the former does not show up in behavioral tests [76].

The confounding contribution of hyperimpulsivity on animals' behavior can be extended to anxiety. As it has been mentioned earlier, several studies found that animals with MTLE present with reduced, rather than increased anxiety (with the interpretation that seizures may have anxiolytic effects) [17, 70]. However, the same animals that showed reduced anxiety in the EPMT presented with hyperimpulsivity in the LRTT [76]. It is thus more plausible that hyperimpulsivity impaired animals' ability to adequately perform in the EPMT for the examination of epilepsy-associated anxiety, thus rendering the test inappropriate under certain conditions.

These observations emphasize the importance of a broader approach in analyzing neurobehavioral disorders in animal epilepsy models. For example, it is not sufficient to merely claim that an animal has deficient spatial or object memory without objective confirmation of the substrate (e.g., dysfunction of place cells and neurodegeneration in perirhinal cortex, respectively). Indeed, if the same animal has depressive impairment (again correlating with the underlying neurobiological

substrate, such as dysfunction of serotonergic transmission or dysregulation of the HPA axis), the lack of motivation may negatively affect its performance in memory tasks.

An effective way to improve clinical relevance of animal models of epilepsy comorbidities would be moving away from formal and toward material models. The latter attempts to account for many interconnected aspects and variables of an analyzed system, and to more closely approximate real-life situations (as Rosenblueth and Wiener put it, "the best material model for a cat is another, or preferably the same cat" [115]).

Material models are preferred even when only one single comorbidity is examined. On the one hand, this would allow accounting for whether and how comorbidities influence one another, and on the other hand, this may help explaining seemingly paradoxical findings by putting them in the context of a multifactorial system.

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