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## A companion to the preclinical common data elements on neurobehavioral comorbidities of epilepsy: a report of the TASK3 behavior working group of the ILAE/AES Joint Translational Task Force

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### SUMMARY

The provided companion has been developed by the Behavioral Working Group of the Joint Translational Task Force of the International League Against Epilepsy (ILAE) and the American Epilepsy Society (AES) with the purpose of assisting the implementation of Preclinical Common Data Elements (CDE) for studying and for reporting neurobehavioral comorbidities in rodent models of epilepsy. Case Report Forms (CRFs) are provided, which should be completed on a per animal/per test basis, whereas the CDEs are a compiled list of the elements that should be reported. This companion is not designed as a list of recommendations, or guidelines for how the tests should be run—rather, it describes the different types of assessments, and highlights the importance of rigorous data collection and transparency in this regard. The tests are divided into 7 categories for examining behavioral dysfunction on the syndrome level: deficits in learning and memory; depression; anxiety; autism; attention deficit/hyperactivity disorder; psychosis; and aggression. Correspondence and integration of these categories into the National Institute of Mental Health (NIMH) Research Domain Criteria (RDoC) is introduced. Developmental aspects are addressed through the introduction of developmental milestones. Discussion includes complexities, limitations, and biases associated with neurobehavioral testing, especially when performed in animals with epilepsy, as well as the importance of rigorous data collection and of transparent reporting. This represents, to our knowledge, the first such resource dedicated to preclinical CDEs for behavioral testing of rodents.

**KEY WORDS:** Animal models, Behavior, Comorbidities, Epilepsy.

## KEY POINTS

- Procedures for assays are summarized to accompany preclinical CDEs on neurobehavioral comorbidities of epilepsy
- Categories include cognitive deficits; depression; anxiety; autism; attention deficit/hyperactivity disorder; psychosis; and aggression
- Test limitations, biases, neurodevelopmental aspects, and optimization of experimental design for epilepsy research are discussed

### A. Mazarati

Neurobehavioral disorders are frequent comorbidities of epilepsy. In addition to having detrimental effects on the patients' quality of life, comorbidities may exacerbate the course of epilepsy, worsen its prognosis, and are frequently associated with refractoriness to antiepileptic drugs.<sup>1</sup> Laboratory studies are instrumental in understanding mechanisms of neurobehavioral comorbidities of epilepsy, and for offering platforms for pre-clinical therapy trials.

Our descriptions of behavioral tests are not intended to serve as a comprehensive manual, but rather as a quick reference guide to be used in association with the Common Data Elements (CDEs).<sup>2</sup> It is our hope that they will assist those investigators embarking on the exploration of epilepsy comorbidities, with study design and logistics, proper experiment planning, and data analysis.

Several methodologic and conceptual limitations should be considered.

**1** The tests are applicable only to rats and mice. Most of the tests have been used in association with epilepsy models in these species; those assays, for which no epilepsy-related reports have been found, are denoted in tables by asterisks (however, given the abundance of sources, it cannot be stated with certainty that such studies have not been performed).

2. Unless specified, the tests are described for adult subjects. Earliest ages for which a behavior of interest has been reported are included in the tables (although the reports are at times serendipitous and should be taken with caution). Methodologies optimized for immature animals are not always congruent with adults—when the differences are substantial, relevant references are provided in the respective sections.
3. Test details, such as duration, setup configurations, test variations, doses of chemicals, and eligible ages, are highly flexible. The provided descriptions are based on the literature, as most commonly used, but should always be validated and adapted by each laboratory to fit its specifics and experimental needs. This is particularly important when considering species, strain, sex, and age (throughout the lifespan, from early to late) of the subjects.
4. Recurrent seizures are a common confounding factor for behavioral testing. Because seizures can hardly be avoided for most epilepsy models, it is important to have detailed seizure records in conjunction with each test. The report of the EEG CDE working group of the ILAE/AES Joint Translational Task Force in this special issue deals exclusively with seizure-monitoring methodology.<sup>3</sup> If EEG monitoring is conducted to document seizures, surgical intervention is required to implant electrodes, and so a sufficient amount of recovery time should be allowed following surgery before behavioral testing commences. It should be considered also that the electrode implantation may damage brain structures involved in the given behavior. In addition, control subjects should undergo the surgery and monitoring. Ideally, one would document seizures before, during, and after behavioral testing is complete. Seizures occurring during habituation and/or training phases of the tests are likely to affect animal's performance during the tests proper. It is ultimately up to the investigator to decide how long the animal should be seizure-free in the run-up to the test, but it is important to document and record this for transparency. In addition, seizures occurring during

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the tests most certainly skew behaviors and respective measures. However, for many tests, video–electroencephalography (EEG) monitoring during testing is not appropriate or not possible, so consider video monitoring at these times to observe for convulsive seizures.

5. Intact basic motor and sensory functions are prerequisites for many tests.<sup>4,5</sup> For example, impaired balance and coordination may confound interpretation of results in swimming tasks (such as the Morris Water Maze and Forced Swimming Test); anosmia may affect performance in such tests as social novelty, and the attentional set-shifting task; and animals in pain or discomfort may not interact socially. Although it may be practically impossible to subject each animal to comprehensive examination of basic functions, this should at least be considered during the initial validation stage in conjunction with the concrete epilepsy model.
6. It is highly likely that the animal with epilepsy develops multiple behavioral impairments, not only those that are the focus of the investigation, and most behaviors are interdependent. For example, all tasks relying on positive and negative reinforcement presume intact memory of a specific type; therefore, impaired cognition may affect animals' performance in unrelated tests (e.g., those for attention-deficit/hyperactivity disorder [ADHD], autism, and anxiety). In a similar vein, adequate performance in these tests requires preserved motivation; hence, the presence of anhedonia may non-specifically affect outcome measures.

Furthermore, specific behavioral tasks may be accomplished successfully by employing alternative strategies. This should be kept in mind, as a loss of cognitive ability may paradoxically facilitate performance by reducing the decision processes for which strategy should be used. Similarly, good performance scores after neuronal injury could be products of alternative strategies that depend on the intact parts of the brain. Disentangling concurrent comorbidities, as well as separating compensatory adaptive events from primary pathologic ones may be difficult, if possible at all. From the comorbidity standpoint, it may be instructive for the subjects to be evaluated on the syndrome, rather than on the symptom level; the presence of several perturbations relevant to the disorder of interest may afford more reliable interpretation (e.g., the poor social interaction, social communication, and restricted behavior present in the same animal provide stronger case for autism-like impairments than poor social interaction alone). By the same token, employing several tests that examine the same dysfunction (e.g., elevated plus maze AND open field test to study generalized anxiety) may enhance the reliability of the findings. In addition, many tests cross several behavioral domains. For example, the elevated plus maze and open field tests are reliant on the same behavioral processes. These tasks both pit the desire to explore a novel space against the fear of open spaces. Other tasks, such as operant conflict tasks or

unconditioned tasks (predator odor exposure) may provide additional information supporting a conclusion of increased or decreased anxiety-like behavior in a task-demand-independent manner.

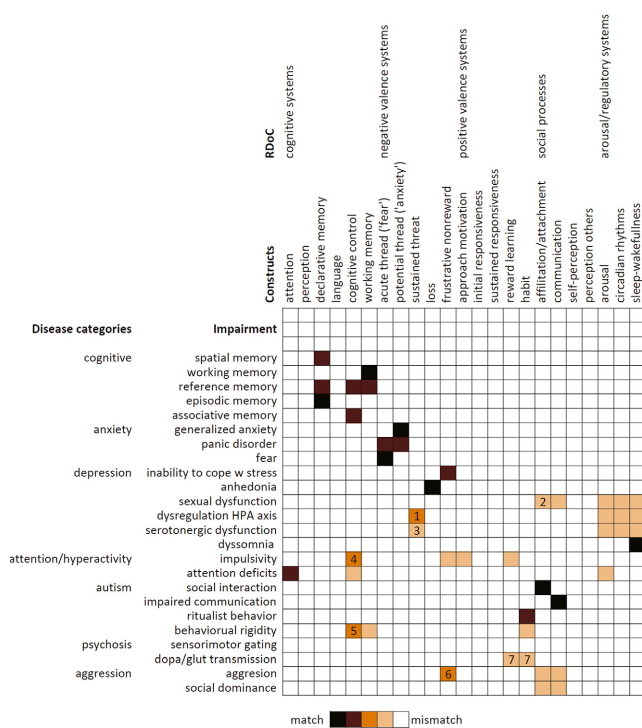
However, subjecting the animal to multiple tests should be undertaken with caution. If possible, the tests should be performed in sequence, rather than in parallel, and should be separated by adequate periods of recovery to avoid the modification of an animal's behavior by preceding tasks. Furthermore, less-taxing tests should be performed first (e.g., for depression studies, taste preference test should precede forced swimming task).

7. A note on terminology: although for practical purposes the narrative uses terms like “depression,” “anxiety,” “ADHD,” and “autism,” etc., it should be appreciated that these conditions are uniquely human constructs, not directly applicable to the animals. It is more correct to refer to the discussed behavioral impairments as “depression-like,” “anxiety-like,” and so on.
8. It is advisable that researchers consider following sound laboratory practices, including, for example, validation of test equipment preferably using the same species, strain, and sex of the test subjects, appropriate sample size calculations, randomization of treatment interventions, and blinding of experimental groups.<sup>6</sup> This latter point is critically important when subjective outcomes are measured, such as some of the behaviors described later. Wherever possible, video-tracking with automated scoring methods should be used to avoid any bias.

## RESEARCH DOMAIN CRITERIA (RDoC) VERSUS NOSOLOGIC APPROACH

### L. M. de la Prida

From a disorder-driven point of view, we consider deficits as belonging to 7 disease categories for examining dysfunctions on the syndrome levels: cognitive deficits (including learning and memory); anxiety; depression; attention-deficit/hyperactivity disorder; autism; psychosis; and aggression. These categories correspond roughly to some RDoC components defined by the National Institute of Mental Health (NIMH; <https://www.nimh.nih.gov/research-priorities/rdoc/index.shtml>):<sup>7,8</sup> cognitive systems; negative valence systems; positive valence systems, and social processes (Fig. 1 black and dark brown cells around the matrix diagonal). Each of our categories sub-classifies several impairment elements, such as deficits of working memory (cognitive deficits), panic disorders (anxiety), dysomnia (depression), impulsivity (attention-deficit/hyperactivity disorder), ritualist behavior (autism), dopaminergic/glutamatergic transmission dysfunction (psychosis), and social dominance (aggression) that match with RDoC constructs in different degrees (Fig. 1; more disperse color-coded cells). For instance, deficits of working memory,



**Figure 1.**

Correspondence between disorders and RDoC constructs. The matrix shows comparison between the TASK3 elements and RDoC constructs as assessed by the degree of matching between the descriptors. Perfect matching is indicated in black; nonoverlapping descriptors are white. 1, dysregulated HPA axis enters as element of Physiology in this construct; 2, sexual dysfunction enters as communication and affiliation in Social Processes and as sex generically in Regulatory Systems; 3, serotonergic dysfunction enters as element of Molecule; 4, impulsive behaviors are included in the sub-construct Response Selection; Inhibition/Suppression; 5, behavioral rigidity enters as the sub-construct Flexible Updating; 6, Physical and Relational Aggression is included in this construct; 7, Dopaminergic/glutamatergic enters as elements of the unit Molecule in Reward Learning and Habits. (L.M. de la Prida).

*Epilepsia Open* © ILAE

episodic memory, fear, and anxiety all match well with the corresponding RDoC constructs in the cognitive and negative valence systems. In contrast, impairment elements for depression, attention/hyperactive deficits, autism, psychosis, and aggression exhibit differing degrees of correspondence. For instance, a dysregulated HPA axis fits better as an element of Physiology in the sustained thread construct (Element 1 in Fig. 1); or impulsive behavior is included in the sub-constructs Response selection; Inhibition/suppression of Cognitive control (Element 4 in Fig. 1; see also caption). This reflects perfectly the multidimensional character of the RDoC framework. RDoC seeks to integrate many levels of information, from genes to behavior, into varying degrees of dysfunction such that diseases can be conceptualized as symptom clusters falling along multiple dimensions as measured by different classes of

units (variables). Other working groups have also conceptualized similar disease categories in terms of the RDoC framework: autism,<sup>9</sup> ADHD,<sup>10</sup> depression,<sup>11</sup> and panic.<sup>12</sup> EEG and electromyographic (EMG) monitoring are implicitly included in the RDoC matrix as elements of the unit Physiology.

## GENERAL EXPERIMENTAL SETTINGS

### A. Mazarati

[File name: 1 General\_Settings CRF; 1 General\_Settings CRF]. Detailed documentation of all experimental settings (i.e., both general and test-specific) is of utmost importance for all behavioral tests. First, this is required to meet the regulations set by the National Institutes of Health (NIH) on research rigor and transparency,<sup>6</sup> as well as to follow the ARRIVE (Animal Research: Reporting of *In vivo* Experiments) guidelines set by the NC3Rs initiative.<sup>13</sup> Second, this enhances the effectiveness of collaboration among different research groups by helping to standardize and coordinate study design. Third, this would help explain commonly encountered disparities in research findings. Even minimal differences in research settings (e.g., time of the experiment, or the duration of handling) may affect outcome measures. The ability to compare the setups used by different laboratories may explain why seemingly identical experimental procedures often produce disparate outcomes. Table 1 provides a nonexhaustive list of preclinical Common Data Elements (CDEs) for reporting general experimental settings, common to all neurobehavioral tests. These CDEs are invariably used in conjunction with Core CDEs,<sup>14</sup> which include individual animal data (e.g., species, strain, date of birth, sex, and source), and on demand, with other relevant CDEs (e.g., Pharmacology,<sup>15</sup> Physiology,<sup>16</sup> and EEG<sup>3</sup>), depending on the experimental goals.

## COGNITIVE IMPAIRMENT

### L. M. de la Prida, P-P Lenck-Santini, N.C. Jones, A. Mazarati

#### *Morris water maze*<sup>17</sup>

[File name: 5 Memory-MWM CRF; 5 Memory-MWM CDE Chart]

**Rationale.** Morris water maze (MWM) is the best-validated and most commonly used test for examining spatial learning and memory (Table 2). The latter is predominantly driven by the hippocampus; hence, hippocampal dysfunction leads to poor performance in the MWM. The test can be used for examining several types of memory (e.g., spatial, working, and reference), and also non-memory functions, particularly behavioral flexibility; as such, it can be used for autism- and schizophrenia-related studies.

**Table 1. Reporting of general settings for behavioral tests**

Common data element	Quantifier
Date	Day, Month, Year
Start/end time	Zeitgeber h:min – h:min
Room temperature	°C
Light-dark cycle type	Normal/reversed
Light-dark cycle: Light phase	Standard h:min
Light-dark cycle: Dark phase	Standard h:min
Area illumination	Lux
Group housing	Number in the cage
Environmental enrichment	Yes/No
Food deprivation prior to the test	Days
Food deprivation during the test	Yes/No
Food restriction prior to the test	Days
Food restriction target weight	% of baseline
Food restriction during the test	Yes / No
Water deprivation prior to the test	Hours
Water deprivation during the test	Yes / No
Water restriction prior to the test	Hours
Water restriction during the test	Yes / No
Handling: number of days	Days
Handling: number of sessions per day	Number
Handling: session duration	Min
Seizure monitoring prior to the test	EEG/video
Seizure monitoring during the test	EEG/video
Seizures detected during the test	EEG/video
Video recording	Yes/No
Video-recording equipment	Name, City, Country, Model name, Model number
Data acquisition equipment	Yes/No
Data acquisition equipment manufacturer	Name, City, Country, Model name, Model number
Software type	Acquisition/analysis
Software developer	Name, City, Country
Software name, version, platform	Name
Test completed	Yes/No

**Procedure.** The apparatus is a large cylindrical tank filled with water to such height that the animal does not touch the bottom when the head is above the water level. The tank is divided into 4 virtual quadrants, conditionally named North, South, East, and West (no actual alignment is required). In the center of one of the quadrants there is a platform, sufficient to accommodate the animal, and submerged under the surface, so that it is invisible to the animal during swimming (except for the familiarization phase, see below). The platform can be further camouflaged by placing an opaque substance in the water (e.g., tempera paint) so that it is also invisible when the animal is diving. The room should have at least one static visual cue placed on each wall (the cues do not have to be intentional; e.g., an entrance door can be a cue as long as it is easily discernable from the wall).

The test included several steps, some of which are mandatory (acquisition and probe tests), whereas others are optional, depending on the goals of the experiment. The test always begins with acquisition, which measures spatial learning, during which the test animal is repeatedly placed

in the tank from different starting locations and is allowed to swim until it finds the platform; there is always a cut-off time, after which if the animal does not locate the platform, it is guided to it manually. The release quadrant should be random across days and should be counterbalanced for the distance away from the platform across the day as well; this is required to prevent the animal from developing motor learning strategy to locate the platform and this may diminish the dependence of the animals' performance on spatial memory. After several days, the time between placing the animal in the tank and reaching the platform should decrease. Acquisition is followed by the probe test, which examines reference memory, whereby the platform is removed, and the time that the animal spends in the quadrant presumably containing the platform is recorded.

Optional tasks include spatial reversal to examine working memory when the platform is moved to a different quadrant, and the ability to learn new quadrant location is detected (this task can also be used for studying behavioral flexibility under autism and schizophrenia protocols). The task can be made more complex to reveal more subtle impairments, to include either spatial double reversal, or repeated learning. Visual discrimination learning involves 2 visible platforms (i.e., elevated over the water level), with easily discernable characteristics (e.g., white and black). One of the platforms is stable, and another is floating, held by a tether. In this task, the animal is expected to learn which platform can be used for the escape.

The test is often preceded with familiarization (also referred to as cued trials), whereby the platform is risen above the water level so that it is visible. This familiarizes the animal with the procedure in general, and facilitates swimming performance. The behavior during the familiarization phase can be used as a control measure to identify differences in swimming speed, motivation to get out of the water, vision impairments, or other factors that may confound the interpretation of differences in acquisition and probe trials. Presumably, if animals are performing at the same level at the end of a session of cued visible platform trials, differences in time/path length to locate the submerged platform depends on spatial memory performance.

For testing in pups, see Ref. 18.

**Analysis and interpretation.** Delayed ability/inability to learn the location of the platform during the acquisition is an indicator of deficient spatial learning; in the probe trial, diminished preference toward the quadrant where the platform was previously located is interpreted as an indicator of deficient reference memory. Delayed learning in reversal tasks is an indicator of behavioral rigidity, and in the discrimination task, an indicator of deficient visual discrimination learning. Successful completion of the acquisition task is a prerequisite for all other subsequent tasks. Animals that are unable to learn the location of the platform are identified as non-performs and are not eligible for further tests.

Table 2. Memory and cognition

Examined impairment in:	Test	Main indicator of the deficit	Youngest reported age	Examples of main settings
Spatial memory	Morris Water Maze (MWM)	Delayed/absent ability to learn location of the platform (spatial acquisition paradigm)	17 days	Dimensions, diameter x height. Rats- 200 cm x 50 cm; mice- 120 cm x 50 cm. Water temperature 24°C. Platform. Size 10 cm <sup>2</sup> . submerged 1 cm below water level. Trials. Duration 60–120 s; intertrial interval 15 s; number per day, 4–6.
	Barnes Maze (BM)	Delayed/absent ability to learn the location of the escape box (acquisition paradigm)	16 days	Dimensions, diameter x height. Rats- 120 cm x 70 cm; mice or immature rats 90 cm x 50 cm Escape hole diameter. Rats- 10 cm; mice or young rats- 5 cm Escape tunnel, diameter x length. Rats- 10 cm x 70 cm; mice or young rats- 5 cm x 50 cm Target box dimensions, Rats- W x L x H. 30 cm x 25 cm x 20 cm; mice or young rats- 25 x 20 x 20 cm Trials. Duration 3–5 min; intertrial interval 15 min; number per day 1–4. Number of arms 8–12. Arm dimensions, length x width. Rats- 50 cm x 10 cm; mice- 30 cm x 5 cm. Food restriction- target weight 85% of baseline Chamber dimensions ≈ 50 cm x 50 cm x 50 cm White noise 85 dB Food deprivation, target weight 85% of baseline. Water deprivation (if water is the reward)- 20–22 h Y-maze dimensions. Start area width x length: 9 cm x 8 cm; goal area width x length: 12 cm x 21 cm. Habituation-test interval 14–16 h. Number of trials 25. T-maze dimensions, length x width x height Start arm: rat 50 cm x 16 cm x 30 cm; mouse 30 cm x 10 cm x 20 cm. Goal arm: rat 50 cm x 10 cm x 30 cm; mouse 30 cm x 10 cm x 20 cm. Food well diameter: rat 2 cm; mouse 1 cm Food restriction, target weight: 85% of baseline See MWM above
Working memory	Radial Arm Maze (RAM)	Repeated entries in the same arm.	23 days	See RAM above
	Match-to-sample/nonmatch-to-sample in adults (MS-A)	Inability/delayed ability to choose operant rewarding behavior	28 days	Apparatus dimensions, diameter x height: 76 cm x 50 cm.
	Match-to-sample/nonmatch-to-sample in pups (MS-P) <sup>a</sup>	Inability/delayed ability to choose the arm in the Y-maze which contains the dam	18 days	
Reference memory	Spatial alternation task (SAT), spontaneous	Decreased ability to learn which arms of the maze contain the reward.	18 days	
	Morris water maze (MWM)	Delayed/absent ability to learn platform location, when the latter is changed daily (spatial working memory paradigm)	21 days	
	Radial Arm maze (RAM)	Failure or delay in learning which arm contains the reward	23 days	
	Place reference (PP)		12 days	

Continued

Table 2. Continued.

Examined impairment in:	Test	Main indicator of the deficit	Youngest reported age	Examples of main settings
		Decreased number of entries into, and/or time spent in the zone/place, where the reward is dispersed.		White noise: 70 dB. Habituation: 3 days, 3 sessions per day, 15 min each. Minimal time in the goal zone 2 s. Steps 1 and 2–3 days each; Steps 3 and 4–8 days each. See SAT above
	Spatial alternation (SAT), rewarded	Decreased ability to learn which arms of the maze contain the reward.	18 days	Arena, diameter 80 cm, elevation 1 m. Electric current duration 0.5 s, intensity 400–500 $\mu$ A.
	Active place avoidance task in carousel (APA)	Failure or delay in learning of the sector where the electric shock is administered	50 days	See MWM above
	Morris water maze (MWM)	Reduced time / distance spent in the target quadrant (probe trial)	21 days	See BN above
	Barnes maze (BN)	Increased latency / path to reach the target hole (probe trial)	16 days	See BN above
Recognition memory	Simple object recognition (SOR)	Lack of preference towards novel vs. familiar object	20 days	Open field dimensions: see Table 3, Anxiety, OFT. Object dimensions: comparable to the subject size. Number of objects- 2–6
	Context object recognition (COR)	Lack of preference towards familiar object, when it appears in a novel context	20 days	
Associative memory	Contextual and Cued Fear Conditioning (CCFC)	Shortened freezing time upon the exposure to the conditioned cue stimulus	17 days	Tone amplitude 60–120 dB; tone duration 15–30 s. Shock current: 0.1–3 mA; shock duration 1–2 s. Inter-trial interval 1–3 min. Number of trials $\geq$ 2. Training-cue/context interval 24 h. Cue-context interval 30 min. Tone-shock overlap (delay version) 1–2 s; tone-shock gap (trace version) 2–60 s. Observation 1–5 min.
Episodic-like memory	What-Where-When & What-Where-Which versions of object recognition	Unbiased object exploration as compared with control animals (not different from chance level).	45 days	Open field dimensions: see Table 3, Anxiety, OFT. Object dimensions: comparable to the subject size. Number of objects- 2–6

<sup>a</sup>No literature records were found on applying this, or similar tests in association with epilepsy models.

*Barnes Maze*<sup>19</sup>

[File name: 2 Memory-BM CRF; 2 Memory-BM CDE Chart]

**Rationale.** The concept of the Barnes maze is similar to that of the Morris water maze. It can be considered a dry version of the latter, and may be more appropriate for use with mice, since mice are not natural aquatic animals. The test examines spatial learning and memory but can be adapted to assess more advanced domains such as cognitive rigidity and working memory. The primary advantage of Barnes maze over the Morris water maze is that it does not rely on the swimming ability; therefore, animals that are not physically fit for swimming may be interrogated for cognitive deficits.

**Procedure.** The maze consists of a large circular platform with several (12–20 depending on the size of the animal) escape holes located near the periphery. One of the holes is connected to a dark escape box, whereas the others lead to false bottoms. Visual cues around the room provide spatial information, and bright overhead lighting provides mildly aversive stimuli to motivate the animal to learn and to remember the location of the escape hole that leads to the dark box. At the start of the test, the test animal is familiarized with the entry into the target box. Then the animal is repeatedly placed at a predetermined position of the maze and is required to learn the escape route. The placement position should be random across days; this is required to prevent the animal from developing motor learning strategy to locate the platform. On subsequent trials, the escape latency is reduced if spatial learning is intact. Similar to the water maze, other cognitive domains can be tested in the Barnes maze, as described earlier. However, these additional tests cannot be performed if the animal is unable to complete the initial spatial navigation task.

**Analysis and interpretation.** Impaired spatial learning is indicated by the increased distance traveled or time spent to locate the escape hole, and/or by an increased number of errors. Search strategies (i.e., random, serial, and direct) can also be assessed, and inform about spatial learning ability. Other behaviors during trials are also recorded, including the patterns of moving in the maze (e.g., moving in the periphery vs. crossing the center; circling). These additional patterns can be useful in identifying abnormal behaviors (e.g., excessive circling in certain animals with neurological deficits or with autism-like features).

*Radial arm maze*<sup>20</sup>

[File name: 7 Memory-RAM CRF; 7 Memory-RAM CDE Chart]

**Rationale:** This task takes advantage of the natural ability of rodents to recruit optimal exploratory strategies for foraging—an essential survival strategy for the species. Here, spatial working memory is operationally defined as

information that is used during the task, whereas reference memory is information that is useful across different exposures to the task. A variety of different paradigms can be adopted, primarily assessing reference memory and/or working memory.

**Procedure:** The test animal is food restricted to motivate them to search for food. The animal is habituated to the apparatus, which consists of a raised maze with 8 arms radiating from a central platform. The central area has gates that can be opened to allow access to one or more arms, and which can confine the animal when appropriate. Spatial cues surround the maze. The animal is placed on the central platform and allowed to explore for food scattered along the arms (first days) and then only at the end of the arms. In the reference memory assessment, the arms are always open, and all arms are baited at the beginning of the trial. Animals are freely allowed to explore the maze and rewarded when they visit a previously unvisited arm. Over time and subsequent trials the animal learns to sequentially visit all the arms without re-entering an arm previously visited. The rate of acquisition of the task for this free-foraging version is an indicator of spatial reference and/or working memory.

In the working memory version, the session starts by placing the animal at the central platform with all doors closed and all arms baited. Central doors are then raised and the animal is allowed to explore until they enter one arm. The remaining arms are then closed. Upon the animal's return to the central platform, the visited arm is closed. The animal is then confined to the central platform for a delay period (the longer the delay, the more taxing the task) before all doors are opened again, and the animal chooses another arm to explore. Only if it chooses a different arm from that previously chosen is the animal rewarded, so it uses the cues around the room to spatially navigate the maze. Sessions are repeated until performance is stable at a given level. This protocol is primarily sensitive to working memory function.

Working and reference memory function can be dissociated by running a second variation of the task. In this version only 4 arms are baited, and the location of these remains constant. If applied after the previously described training, the animal is allowed to explore the entire maze to retrieve all 4 rewards. In a next session, the same 4 arms are baited and the animal is again allowed to explore. The sequence is repeated until accuracy reaches an upper level for both memory modalities (entries into unbaited arms are reference memory errors; re-entries into baited arms are working memory errors).

For testing in pups, see Ref. 21.

**Analysis and interpretation:** Behavior is measured by assessing the number of correct and incorrect entries, depending on the protocol used. The time taken for animals to retrieve all rewards or to complete a given task at a particular accuracy level (typically >85%; but it could be different for experimental groups) is also informative. Working memory errors within a given session are evaluated by the

number of incorrect arm choices. To evaluate working and reference memory errors at once, entries into baited arms (working memory errors) and unbaited arms (reference memory errors) are evaluated.

#### *Match/non-match to sample—adults*<sup>22</sup>

[File name: 3 Memory-MS-A CRF; 3 Memory-MS-A CDE Chart]

**Rationale.** Derived from delayed-response principles, the task is a widely used test of working memory in animals.

**Procedure.** The test animal should be initially food restricted and habituated to the specialized operant chamber. The task involves paired sample and choice phases. In the sample phase, the animal is presented with one of 2 levers, which the animal needs to press to receive a reward (typically food pellet). After a delay, the choice phase is initiated, and the animal is presented with 2 levers. Pressing only one of the levers will result in a reward, so it has to choose one or the other lever successfully. In the match-to-sample version, the food reward is delivered only if the animal presses the same lever as the one before, whereas in the non-match-to-sample version, the animal must press the different lever. The paired trials are repeated several times within a session, and the tasks require extensive training.

**Analysis and interpretation.** Working memory performance is measured as the percentage of correct compared to total responses (50% being random performance).

#### *Non-match-to-sample—pups*<sup>23</sup>

[File name: 4 Memory-MS-P CRF; 4 Memory-MS-P CDE Chart]

**Rationale.** This version of the task is run under a similar pretext as the adult version, except rodent pups are motivated to remember the location of an anesthetized lactating dam.

**Procedure.** The task is conducted in a Y-maze, with paired forced/choice phases. In the forced phase, the lactating dam is at one arm of the maze, and the pup only has access to this arm. After a variable delay, the choice phase occurs, with the pup offered access to both arms. For the non-match version, the dam is located in the alternate arm, and if the pup correctly chooses this location, it is allowed to suckle on the exposed nipple.

**Analysis and interpretation.** As for above, the number of correct vs total trials are recorded, with 50% success being chance level.

#### *Spatial alternation task*<sup>24</sup>

[File name: 8 Memory-SAT CRF; 8 Memory-SAT CDE Chart]

**Rationale.** The spatial alternation task is a simpler version of the radial arm maze. Spatial working memory and

reference memory can be challenged using either spontaneous or rewarded version of this task.

**Procedure.** A T-shaped maze is typically used, consisting of a start area connected directly to the central arm and 2 goal arms that radiate from a central zone. Guillotine doors in the start area control the delay between trials, and a barrier at the choice region restricts access to one arm at a time. For the rewarded alternation version, the test animal should be food-restricted, and habituated to the maze and to food rewards in the goal arms. The task consists of paired sample and choice trials. For the sample trial, both arms are baited but the choice arm is blocked by a barrier. The animal is placed into the start area and is allowed to move in the maze and to consume food in the sample arm. The animal is then returned to the start position for a variable amount of time. The longer this delay, the more taxing the task. Access to both arms is then offered, but only the choice arm is baited. The animal explores the maze and freely chooses one arm. It needs to remember the previous location of the sample arm and alternate its choice to receive the reward. The process is repeated in several trials per session. For the spontaneous alternation version, the animal is neither habituated nor food-restricted, as it is the novelty of the maze that drives the spontaneous alternation. In this instance, the food reward is alternated between arms, and so by alternating arm choices, the animal successfully receives its reward.

For testing in pups, see Ref. 25.

**Analysis and interpretation.** The number of correct (alternate) vs. incorrect (repeated entry into previously chosen arm) responses is used to evaluate an animal's performance. For the rewarded version, animals are typically trained to an a priori defined criterion (e.g., >80% accuracy); the number of sessions/trials required to reach criterion is also evaluated. For the spontaneous alternation version, no criterion is set, and the percentage of correct entries is calculated,

#### *Place preference*<sup>26</sup>

[File name: 6 Memory-PP CRF; 6 Memory-PP CDE Chart]

**Rationale.** The test animal is required to use spatial information to navigate to a goal area in a circular maze. The motivation can be aversive (e.g., bright light, noise) or rewarding (e.g. food reward).

**Procedure.** The animal is trained to reach an unmarked location in the environment in order to obtain a food reward, or to stop the occurrence of noxious stimuli, such as bright light and noise. The goal is identifiable by the spatial relationships it shares with the visual cues present in the periphery.

For testing in pups, see Ref. 27.

**Analysis and interpretation.** Number of entries to the target zone compared to a neutral, equal size zone in the apparatus, is indicative of learning. For mice, the aversive version is easier to implement.

*Active place avoidance task in carousel*<sup>28</sup>

[File name: 1 Memory-APA CRF; 1 Memory-APA CDE Chart]

**Rationale.** Using a rotating circular arena, the test animal learns to associate a section of the arena with a noxious stimulus. This is achieved via the use of spatial cues.

**Procedure:** The active place avoidance task is analogous to the place preference task, except it is typically motivated by aversive stimuli. A circular arena is equipped with a section of the base wired to deliver a mild foot shock. High-contrast visual cues around the room and on the floor of the arena indicate this avoidance zone. The animal has to associate the visual cues with this zone and learn to avoid it. Failure to do so will result in a mild electric foot shock, unpleasant to the animal.

**Analysis and interpretation:** Measures consist of number of entrances (spatial abilities), number of shocks received (estimating motivational state to escape the shock), and time to first entrance to the shock zone (estimating between-session memory).

*Simple object recognition*<sup>29</sup>

[File name: 9 Memory-SOR CRF; 9 Memory-SOR CDE Chart]

**Rationale.** Object recognition (OR) tasks are based on the relative exploration of a novel object versus a familiar one. There is no necessity of learning any rules, or any motivating elements (e.g., appetitive or reward) and is relatively quick and easy to administer (although a natural investigative motivation, or lack thereof due to epileptic state, should not be discounted). Several variations of the simple task exist, which may challenge different mnemonic processes, including spatial, object, object-location, and temporal memory (or recency).

**Procedure.** The single object recognition task is a 2-trial test. The test animal is initially habituated to the testing environment, an open arena. Then, in the first trial, the animal is entered into the arena, which now contains a pair of identical objects that they explore and interact with for 5 min. They are then removed for the intertrial interval and placed in the home cage for a variable length of time. In the test phase, the animal enters the arena to encounter some novelty. One of the now-familiar objects has been replaced with a novel object of about the same dimensions. Alternate versions of the task can move one of the familiar objects to a new location. The animal then explores the objects—normal animals will preferentially explore the novel object. Intertrial interval may vary from minutes to hours depending on the experimental design. Simple tasks use 3- or 5-minute intertrial interval and rely on short-memory function. Longer intertrial intervals (e.g., tens of minutes to hours) are typically used to evaluate long-term memory.

**Analysis and interpretation.** Time spent exploring familiar and novel object is recorded. A normal animal will prefer the novel object. If episodic memory is impaired, the animal treats the familiar object as novel, and spends similar time exploring both objects.

*Episodic-like memory*<sup>30</sup>

[File name: 9 Memory-SOR CRF; 9 Memory-SOR CDE Chart]

**Rationale.** Deficits of episodic memory, that is, the memory of autobiographical events (times, places, associated emotions, and other contextual “who,” “what,” “when,” “where,” “which” knowledge) are common in epilepsy. Because episodic memory is related directly with language and consciousness, their study in nonverbal animals and specifically in rodents is controversial. However, different paradigms have been devised to test for episodic-like memory in animals. Thus the specific attributes of an episode are separated into the “what” happens “where,” with contextual information (temporal “when” or circumstantial “which”) being implicitly considered. Today, 2 specific paradigms exploit object recognition tasks to test for integrated memory for “what-where-when” (WWWhen<sup>31</sup>) and “what-where-which” (WWWhich<sup>31</sup>).

**Procedure.** In general, the test is an Object Recognition task with 2 sample phases where the test animal encounters different sets of objects in different arrangements and/or context, followed by a test phase where the animal finds the previous objects in different places/time/context. The interphase interval can vary from a few minutes (5–15 min) to several tens of minutes (50–90 min) or even days (24 h). For the WWWhen tasks, it is recommended to use the version developed by Dere et al.<sup>31</sup> for mice, and the task applied in Inostroza et al.<sup>30</sup> for rats. For WWWhich tasks, researchers can check the design by Easton and colleagues.<sup>32</sup>

**Analysis and interpretation.** Animals will show biased exploration for objects depending on their different “what,” “when,” “where,” and “which” memory load (and combinations). The typical exploratory bias of a control group could depend on the task configuration and on the species. For instance, normal mice and rats differ in their basal exploratory bias in the WWWhen task. Similar to standard OR tasks, the total time an animal spends exploring each object is evaluated, with object exploration defined as the animal being within 2 cm of the object, directing its nose at the object, and being involved in active exploration such as sniffing. The proportion of time the animal spends exploring each object is transformed in a discrimination index to test against chance level or different indices, according to the task design.

*Context object recognition*<sup>33</sup>

[File name: 10 Memory-COR CRF; 10 Memory-COR CDE Chart]

**Rationale.** The rationale is similar to that for the simple object recognition, but the test adds a contextual component. This challenges the animal to associate novel objects with novel contexts in which the objects are presented. The task can be further advanced by varying the location of the novel object—alone or in combination with contextual variation.

**Procedure.** First, the test animal is habituated to the arena on several days, with at least 2 sessions exposed to each different context to be used in the testing phase. Then, each test session consists of 2 exposure phases, followed by a variable delay (2–120 min) and then the test phase. In the first exposure phase, the animal is placed in the arena with one context (e.g., smooth floor) and is exposed to 2 objects that are different in shape. In the second exposure phase, the context is changed (e.g., mesh floor), and the position of the same 2 objects is reversed, thereby associating the new positions of the objects with different context. In the test phase, the first context is used, and the 2 objects are placed in the same locations as before, but the objects are now identical. Varying the objects and the contexts used in the test phase in different animals reduces any bias. Normal animals will recognize which of the objects has been investigated previously in that context, and in which position it was, and will preferentially explore the other object that is in a location with no contextual exposure.

**Analysis and interpretation.** Total time that the animal spends exploring each object is recorded. During the first 2 phases, animal with normal context object recognition should explore the 2 objects equally, while in the test phase the animal would prefer the object that is novel in the context. Failure to do so suggests a deficit in episodic memory.

*Contextual and cued fear conditioning*<sup>34</sup>

[File name: 2 Anxiety-Memory-CCFC CRF; 2 Anxiety-Memory-CCFC CDE Chart]

**Rationale.** Fear conditioning examines the associative learning ability of an animal. It consists of combining a neutral conditioned environment (i.e., the context) and/or a stimulus (i.e., a cue, such as a tone) with an aversive unconditioned stimulus (e.g., foot shock). Fear response in rodents, measured as the amount of time spent immobile (freezing), is an indicator of the animal's ability to associate the unconditioned stimulus to the conditioned stimulus.

Most commonly used experimental design is a 2-trial delay cued and contextual fear conditioning.

**Procedure:** Contextual fear conditioning involves placing the test animal in a novel environment and delivering an unconditioned stimulus (i.e., foot shock). On subsequent exposure to the environment, the animal will exhibit freezing behavior if it remembers that the previous exposure was associated with the foot shock. Cued fear conditioning is similar to the contextual version, but includes a cue, such as an auditory tone. The animal is expected to associate the cue with the shock, and when subsequently exposed to the environment and the cue, the animal will display freezing behavior. Additional delay and trace conditioning paradigms can be incorporated into the cued fear conditioning paradigm, related to the timing of the shock with respect to the tone. Trace conditioning involves inclusion of a time delay between the offset of the tone and the onset of the shock, whereas in delay conditioning, the shock occurs in the presence of the tone. An important methodologic consideration is to acoustically isolate animals that have been aversively conditioned from those who have not, to avoid bystander communication.

In the standard paradigm consisting of a 2-trial delay cue and contextual fear conditioning, on day 1, the animal is habituated for several minutes to the conditioning chamber. Then, on the next day, the animal is re-entered into the chamber, and an auditory stimulus (70–80 dB) is presented for 15–30 s. At the end of this cued period, a mild electric shock (0.17–0.8 mA) lasting 1–2 s is delivered through the floor grid to the animal. The cue and shock co-terminate, which then initiates an intertrial interval of 1–3 min. This cue + shock pairing is then repeated, and the animal is returned to the home cage. On day 2, the animal is placed in the chamber with different contextual aspects (e.g., different lighting, odor, wall patterns). After 2 min of habituation, the animal is exposed to the conditioned stimulus used on day 1, and the resulting behavior is assessed.

For testing in pups, see Ref. 35.

**Analysis and interpretation.** The quantifiable measure is the duration of freezing behavior exhibited by the animal when re-exposed to the same conditions (contextual or cued) previously paired to the shock. The absence or shortening of freezing time would indicate deficits in associative memory. Conversely, increased duration of freezing may be an expression of generalized anxiety, or phobia-like behavior. In the context of epilepsy, it is important to make sure that freezing behavior is not a manifestation of a nonconvulsive seizure, but is indeed a behavioral response to the cue, although this could only be ascertained using EEG during the test. Although fear conditioning in general depends on the integrity of the amygdala, response to context exposure and presentation of the cue after trace conditioning is also hippocampus dependent.

Table 3. Depression

Examined impairment	Test	Main indicator of the deficit	Youngest reported age	Examples of main settings
Inability to cope with stressful situation	Forced swimming (FST)	Increased immobility	21 days	Tank dimensions: diameter $\approx$ 1.5 of the trunk length; height $\approx$ 1.5 of the body length (including tail). Water temperature 24°C. Water level from the rim $\approx$ 1/5 of the trunk length. Drop height $\approx$ 1/5 of the trunk length. Test duration 5 min.
Anhedonia	Tail suspension (TST)  Taste preference (TPT)	Increased immobility  Diminished preference towards sweetened drinks	21 days  21 days	Bar height: 30–50 cm. Tail separation screen: recommended to prevent tail-climbing. Tail fixation point 1–2 cm from the tail base. Habituation 1 min; test proper 5 min. Sucrose 1% or 20% in tap water; saccharin 0.1% in tap water.  Habituation 15 min - short version; 24 h - long version.
Sexual dysfunction	Sexual behavior in males (SEX)	Increased latency and/or decreased frequency of mounting, intromission and ejaculation	60 days	Test duration 15 min - short version; 24 h - long version. Light-dark cycle normal; start-end time Z12-Z24. Light red or infrared. Estradiol injection (female partner) 50 $\mu$ g/kg, 48 h prior to the test; progesterone injection (female partner) 100 $\mu$ g/kg, 6 h prior to the test. Eligibility $\geq$ 3 ejaculations over 3 days during the pre-test. Duration of immobilization: 15–45 min. Blood collection immediately before, and after the immobilization.
Dysregulation of the HPA axis	Endocrine response to the immobilization stress (IMS)	Exacerbated increase of circulating corticosterone (CORT) in response to immobilization	2 days	Blood sample 50 $\mu$ l. DEX: 30 $\mu$ g/kg i.v.; blood collection immediately before, and 6–24 h after the injection. CRH: 30 ng/kg i.v., immediately after post-DEX blood collection; blood collection 30 and 60 min after the injection. Blood sample 50 $\mu$ l. 8-OH-DPAT: 0.1–1.0 mg/kg s.c. or i.p. Vehicle temperature 37°C. Temperature measurements 15, 30, 45, 60 min after the 8-OH-DPAT injection.
Serotonergic dysfunction	Combined dexamethasone-corticotropin releasing hormone test (DEXCRH)  8-OH-DPAT - induced hypothermia (DPAT) <sup>o</sup>	Less pronounced suppression of circulating CORT by DEX; exacerbated increase of circulating CORT by CRH  Exacerbated decrease of body temperature in response to 8-OH-DPAT	50 days  1 day	EEG: Sampling rate 256 Hz; band-pass filter 0.5–35 Hz EOG: Sampling rate 64 Hz; band-pass 0.5–30 Hz EMG: Sampling rate 256 Hz; band-pass 80–100 Hz Duration of monitoring $\geq$ 96 h
Dysomnia	Sleep monitoring (SLEEP)	Shortened latency, increased duration, and increased number of episodes of REM sleep	8 days	

<sup>o</sup>No literature records were found on applying this, or similar tests in association with epilepsy models.

## DEPRESSION

**K. Sarkisova, A. Mazarati**

*Forced swimming test (FST)*<sup>36–39</sup>

[File name: 3 Depression-FST CRF; 3 Depression-FST CDE Chart]

**Rationale.** FST examines the ability of an animal to effectively cope with an inescapable stressful situation.<sup>40</sup> This is created by forcing the animal to swim in an enclosure with no escape options (Table 3). The animal adopts several behavioral patterns, which are interpreted as either adaptive (i.e., escape attempts) or nonadaptive (i.e., no escape attempts).

**Procedure.** The test animal is allowed to swim in a water-filled tank, typically for 5 min.

**Analysis and interpretation.** Two behavioral patterns are most commonly analyzed. (1) Active behavior is evident as escape attempts, such as climbing and swimming along the walls, and diving. In normal subjects, this is dominant behavior, accounting for approximately  $\geq 66\%$  of the test duration. (2) Passive behavior is immobility, whereby the animal is moving only enough to maintain its head above the water but makes no escape attempts. In normal subjects, immobility is present, but typically does not exceed 33% of the total swimming time. The increase in immobility is regarded as an indicator of despair/hopelessness. Other behaviors may also be present. Struggling away from the walls (i.e., active behavior, but with no attempts to escape) is minimally present in normal subjects. Because immobility requires intact motor functions, vestibular abnormalities may lead to the displacement of immobility with such behavior, whereby the animal is struggling to avoid drowning, rather than to escape. Concurrent ADHD-like impairments may manifest as increases in no-escape struggle.<sup>38</sup> Most commonly, the cumulative duration of each behavior is calculated. The number of episodes, and the latency of the immobility can also be considered.

*Tail suspension test (TST)*<sup>41</sup>

[File name: 8 Depression-TST CRF; 8 Depression-TST CDE Chart]

**Rationale** is the same as for the FST. In the TST, an inescapable stressful situation is created by suspending the animal by the tail. The test is more commonly employed for mice than for rats.

**Procedure.** The test animal is suspended by the tail from the horizontal bar for 6 min. Behavior is recorded, starting from the second minute.

**Analysis and interpretation.** Two behaviors are present: struggling, and immobility. Both behaviors are present in

normal subjects with an approximate 50:50 ratio. Increased immobility is the indicator of hopelessness/despair. Cumulative duration and number of episodes of each behavior, as well as the latency to the first immobility episode are recorded.

*Taste preference test (TPT)*<sup>42,43</sup>

[File name: 7 Depression-TPT CRF; 7 Depression-TPT CDE Chart]

**Rationale.** TPT is used to examine anhedonia. The test is based on the inherent affinity of rodents toward sweets.

**Procedure.** For habituation, 2 identical bottles are introduced in the home cage, both filled with tap water, and the test animal is free to drink from either. On the next day, water in one of the bottles is replaced with a sweet drink—either 0.1% saccharin or 1% sucrose. To avoid bias, the position of the bottles (i.e., left or right) should be alternated or randomized between animals. Twenty-four hours later, the volumes of the consumed water and of the sweet drink are recorded. In the short version of the test, which lasts 15 min, 20% sucrose is offered.

**Analysis and interpretation.** During habituation, the volumes consumed from each bottle should be similar; if not, the setup should be checked for biases (e.g., illumination and access to the bottles). During the test proper, normal animals preferentially consume the sweet drink (typical sweet solution: water consumption ratio is  $\geq 2:1$ ). An anhedonic-like state is present if the sweet solution-to-water ratio is  $< 2:1$  and  $> 1:2$ . A ratio of  $< 1:2$  may suggest a taste aversion rather than anhedonia; proper interpretation of such outcome is complicated.

**Analysis and interpretation.** For simple estimate, taste preference is analyzed by comparing the volume of consumed sweet solution against the amount of consumed water. More detailed analysis involves counting the number of approaches to each bottle, with the assumption that normal animals will approach the bottle with the sweet solution more frequently.

*Sexual behavior*<sup>42</sup>

[File name: 5 Depression-SEX CRF; 5 Depression-SEX CDE Chart]

**Rationale.** Depression may be characterized by sexual dysfunction.

**Procedure.** The test is performed in males only, whereby their behavior is evaluated vis-à-vis female partners. (1) Preparation of female partners. Female rats are ovariectomized 2 weeks or more prior to the test following a standard surgical procedure (many vendors can ship ovariectomized animals). Forty-8 h before the mating, females are injected with estradiol benzoate; 6 h before the test animals are injected with progesterone (both

subcutaneously). (2) Mating is carried out during the dark phase of a 12 h light-dark cycle. The room is lit with dim red light. Food and water are removed from the home cage. (3) Selection of males. Males should be sexually inexperienced at the beginning of the experiment. Prior to the experiment, the female rat is introduced into the home cage and the test rat's behavior is recorded. The session is repeated on 3 consecutive days. The males are used for further experimentation if they have a total of 3 ejaculations during the selection. Otherwise, the animals are identified as noncopulators and are not recommended for further studies. (4) Test proper. Female rat is introduced into the home cage for 30 min and the male's behavior is recorded.

*Analysis and interpretation.* Simple indicators of sexual activity are mounting, intromission, and ejaculation, which can be either selectively or globally suppressed in depression. Analyzed parameters: (1) Mount latency: time elapsed between introducing the female and the first mounting trial without intromission; (2) Mount frequency; (3) Intromission latency: time elapsed between introducing the female rat into the male cage and the first intromission; (4) Intromission frequency; (5) Ejaculation latency: time elapsed between the first penetration and ejaculation; (6) Ejaculation frequency.

#### *Combined dexamethasone/corticotropin-releasing hormone (DEX/CRH) test<sup>44</sup>*

[File name: 2 Depression-DEXCRH CRF; 2 Depression-DEXCRH CDE Chart]

*Rationale.* The dysregulation of the hypothalamic-pituitary-adrenocortical axis (HPA-A) is a neuroendocrine hallmark of chronic stress. The phenomenon is defined as a failure of circulating cortisol (or corticosterone [CORT] in rodents) to engage the negative feedback loop, which includes CRH and ACTH, so that the level of circulating CORT becomes unabated. The DEX/CRH test is designed to indirectly gauge the function of the HPA-A.

*Procedure.* The test includes a series of blood collections, and subsequent detection of CORT in plasma. Venous blood samples (approx. 50  $\mu$ l) can be collected either from the femoral vein in freely moving subjects via a pre-implanted catheter, or from the tail vein under anesthesia. The procedure includes the following: (1) baseline blood collection; (2) i.v. injection of DEX (30  $\mu$ g/kg) immediately after (1); (3) blood collection 6 h after DEX injection; (4) i.v. injection of CRH (50 ng/kg) immediately after (3); (5) blood collection 30 min and 60 min after (4).

As a shorter version, the DEX suppression test can be performed in lieu of the combined DEX/CRH test; the test is performed as described, except it is terminated once blood is collected after DEX injection. Blood samples are centrifuged at 4000g at 4°C, plasma collected, aliquoted at 10–20  $\mu$ l and stored at –80°C.

*Analysis and interpretation.* As a normal response to DEX, plasma CORT concentration decreases 2- or more

fold. A normal response to CRH is a moderate increase (to approximately pre-DEX level) of plasma CORT at 30 min, and its return to pre-CRH level at 60 min. A blunted or absent response to DEX, and/or exacerbated and prolonged elevation of CORT after CRH injection are indicators of the hyperactive HPA-A. CORT is detected in plasma by either enzyme immunoassay, or radio-immunoassay, using commercially available kits and standard plate reader. Measurements are done in at least duplicate, for each of the blood collections. The activity of the HPA-A can be expressed either in absolute numbers (ng/ml of plasma) or normalized vs. baseline CORT concentration.

#### *Endocrine response to immobilization stress<sup>45</sup>*

[File name: 4 Depression-IMS CRF; 4 Depression-IMS CDE Chart]

*Rationale* is the same as for the combined DEX/CRH test. The difference is that the HPA-A is stimulated through subjecting the animal to stressful situation (immobilization), rather than by injecting CRH.

*Procedure.* The test animal is placed inside the restraining tube with the tail protruding from one end. A baseline blood sample (50  $\mu$ l) is collected from the tail vein. The animal remains restricted in the tube for 30 min, at which point the second blood sample is collected. Blood samples are centrifuged at 4000g at 4°C, plasma collected, aliquoted at 10–20  $\mu$ l, and stored at –80°C.

For testing in pups, see Ref. 46.

*Analysis and interpretation.* In normal subjects, the immobilization leads to moderate increase of plasma CORT level. The increase is exacerbated under conditions of dysregulation of the HPA-A. The analysis is the same as for the DEX/CRH test.

#### *8-OH-DPAT- induced hypothermia<sup>47</sup>*

[File name: 1 Depression-8OHDPAT CRF LCH; 1 Depression-8OHDPAT CDE Chart]

*Rationale.* Depression has been associated with hypersensitivity of 5-HT<sub>1A</sub> receptors. A selective 5-HT<sub>1A</sub> agonist 7-(dipropylamino)-5,6,7,8-tetrahydro-naphthalen-1-ol (8-OH-DPAT), lowers body temperature when administered systemically. With 5-HT<sub>1A</sub> receptor hypersensitivity, 8-OH-DPAT-induced hypothermia is exacerbated.

*Procedure.* Baseline reading of body temperature (using rectal, infrared, surface, or chronically implanted sensor) is taken and the animal is injected subcutaneously with 8-OH-DPAT. Repeated temperature measurements are taken afterward, typically 4 readings at 15 min intervals. The protocol may include gauging either the response to standard dose of 8-OH-DPAT (e.g., 0.4 mg/kg), or the dose-response, whereby the injections are performed every 48–72 h at 0.1–1.0 mg/kg arbitrary increments (e.g., 0.15 mg/kg).

Table 4. Anxiety

Examined impairment	Test	Main indicator of the deficit	Youngest reported age	Examples of main settings
Generalized anxiety	Elevated plus maze (EPM)	Increased presence in closed arms and/or decreased presence in open arms	14 days	<p>Dimensions: Rat: Arm length, arm width, wall height <math>\approx</math> 50 cm, 10 cm, 50 cm.</p> <p>Center square 10 <math>\times</math> 10 cm.</p> <p>Mouse: Arm length, arm width, wall height <math>\approx</math> 30 cm, 6 cm, 40 cm. Center square 6 <math>\times</math> 6 cm</p> <p>Elevation 50–70 cm above the floor level</p> <p>illumination. Dim configuration: open arms 30–40 Lx; closed arms 5–10 Lx.</p> <p>Bright configuration: open arms 200–400 Lx; closed arms 20–30 Lx</p> <p>Test duration: 5–10 min</p>
	Open field test (OFT)	Fewer visits to, and/or reduced presence in the central area; more visits to and/or increased presence in the peripheral area	12 days	<p>Dimensions: Square: <math>\approx</math> 60 <math>\times</math> 60 cm, or 72 <math>\times</math> 72 cm, or 90 <math>\times</math> 90 cm</p> <p>Circular, diameter 80–100 cm.</p> <p>Number of squares: 16 or 25</p> <p>Wall height 30–50 cm</p> <p>illumination. Dim configuration: peripheral 20–40 Lx; central 40–80 Lx</p> <p>Bright configuration: peripheral 80–150 Lx; central 150–200 Lx</p> <p>Test duration: 5–10 min</p>
	Stress-induced hyperthermia (SIH) <sup>a</sup>	Exacerbated increase in body temperature in response to the transfer stress	35 days	<p>Test duration: 5–10 min</p> <p>Basal temperature reading (before transfer) - 4 readings at 30 min intervals.</p> <p>Time between last basal reading and the transfer: 24 h.</p> <p>Temperature readings after the transfer: 30 and 60 min.</p>
	Novelty-suppressed feeding (NSF)	Failure to consume food when exposed to novel environment	25 days	<p>Food deprivation prior to the test 18–24 h.</p> <p>Open field specifications- see OFT</p> <p>Test duration 10 min</p> <p>Time to consume pre-weighed food upon the return in the home cage - 10 min</p> <p>Electrode coordinates, mm from Bregma. Rat: AP = -7.3; L = 0.6; V = 5.0; Mouse: AP = -4.24; L = 0.5; V = 2.2</p> <p>Post-surgery recovery period <math>\geq</math> 7 days</p>
Panic disorder	Behavioral response to electrical stimulation of Dorsal Periaqueductal Grey (DPAG)	Decreased stimulation threshold for eliciting panic-like responses	65 days	<p>Stimulation parameters. Pulse waveform bipolar square wave; pulse duration 1 ms; inter-pulse interval 16.7 or 20 ms; train duration 30 s; inter-train interval 5 min.</p> <p>Starting stimulus intensity 20 <math>\mu</math>A; increment 5 <math>\mu</math>A; maximal stimulus intensity 100 <math>\mu</math>A</p> <p>See CCFC in Table 1.</p>
Fear	Contextual and Cued Fear Conditioning (CCFC)	Increased freezing time upon the exposure to the conditioned cue stimulus	17 days	

<sup>a</sup>No literature records were found on applying this, or similar tests in association with epilepsy models.

*Analysis and interpretation and analysis.* An exacerbated response to the standard dose of 8-OH-DPAT and/or steeper dose-response slope suggest 5-HT<sub>1A</sub> receptor hypersensitivity.

#### *Sleep structure*<sup>47,48</sup>

[File name: 6 Depression-SLEEP CRF; 6 Depression-SLEEP CDE Chart]

*Rationale.* Depression is characterized by impairments in sleep structure, particularly by the increased presence of the rapid eye movement (REM) sleep.

*Procedure.* An array of electrodes is implanted to record EEG, electro-oculogram (EOG), and electromyogram (EMG). Electrographic and video recording is performed over at least 96 h, preferably in the home cage (or, after at least 1 week habituation in a dedicated chamber), during a normal dark-light cycle.

For testing in pups, see Ref. 49.

*Analysis and interpretation.* Depression-relevant impairments include shortening of REM sleep latency, increased number of REM sleep episodes, and prolonged REM sleep duration. Analyzed parameters: (1) frequency and duration of waking and sleep states; (2) frequency and duration (both individual and cumulative) of slow wave sleep episodes; (3) frequency and duration (both individual and cumulative) of non-REM sleep episodes; (4) latency of REM sleep episodes (i.e., time between sleep onset and the nearest REM sleep episode).

Electrographically, the waking state is characterized by high EMG and low EEG amplitude and high theta activity concomitant with highest EMG values. Non-REM sleep is characterized by low EMG amplitude, high EEG amplitude with high delta activity, and absence of EOG activity. REM sleep is characterized by low EMG and low EEG amplitude, high theta activity, and high EOG activity.

## ANXIETY

**N.C. Jones, J. Veliskova, A. Mazarati**

#### *Elevated plus maze*<sup>50</sup>

[File name: 1 Anxiety-EPM CRF; 1 Anxiety-EPM CDE Chart]

*Rationale.* The test relies on a rodent's inherent preference for enclosed dark spaces versus exposed elevated spaces (Table 4).

*Procedure.* The apparatus is typically cross-shaped maze with 2 opposing closed arms and 2 opposing open arms, elevated above the floor/desk level. The intersection (starting position), is exposed. Illumination of open arms may vary from ambient room light to bright light, the latter used to amplify the exposure to the open space. The test lasts 5–10 min and starts by placing the test animal at

the intersection. The animal is allowed to freely move along the arms.

*Analysis and interpretation.* Rodents always spend more time in the enclosed arms. Reduced presence in the open arms is regarded as an indicator of anxiety. Total time spent in, and the number of entries in the open and closed arms is calculated.

#### *Open Field*<sup>42</sup>

[File name: 4 Anxiety-OF CRF; 4 Anxiety-OF CDE Chart]

*Rationale.* The Open Field has many purposes, including the analysis of locomotor and exploratory activities. In the context of anxiety, the emphasis is on the time spent on the periphery, close to the walls (more secure space) versus central portions (less secure space).

*Procedure.* The apparatus is typically square surrounded by walls. For quantification purposes, a square grid can be drawn on the floor (e.g., 4 × 4 or 5 × 5); holes may be present at the square intersections for the evaluation of exploratory behavior. The area can be illuminated evenly or can be adjusted so that peripheral segments receive less light than the center. The test lasts 5–10 min, started by placing the test animal in the center. The animal is allowed to move freely in the field.

*Analysis and interpretation.* Normal rodents prefer the periphery over the center area; further increase of this preference is an indicator of anxiety. Suppression of exploratory behavior is suggestive of anxiety also. Excessive exploratory behavior as compared to controls may point toward hyperactivity. The most informative parameters are the number of peripheral and central crossed squares; and the time spent in peripheral and central squares. Parameters pertinent to exploratory behavior include total number of crossed squares, number of rearings, and number of hole explorations.

#### *Stress-induced hyperthermia*<sup>51</sup>

[File name: 6 Anxiety-SIH CRF; 6 Anxiety-SIH CDE Chart]

*Rationale.* In rodents, body temperature rises in response to stress; exacerbated rise in body temperature is evidence of anxiety.

*Procedure.* A stressful situation is created by moving the test animal from their usual location. Prior to relocating, body temperature is acquired, typically 2 to 4 times, at 30-min intervals, using rectal, infrared, surface, or chronically implanted sensor. Various transfer paradigms can be used, alone, or in combination. For example, the animal's cage can be moved inside the room from one place to another; cage lids can be temporarily removed, etc. In addition, the animal can be transferred to a different room, for example, via a noisy corridor. Temperature readings are taken several times after the transfer, with at least 2 readings, at 30 and 60 min.

Table 5. Autism

Examined impairment	Test	Main indicator of the deficit	Youngest reported age	Examples of main settings
Impaired social interaction	Three-chamber test (3CH)	Diminished preference towards the conspecific vs. an indifferent object (impaired sociability); diminished preference towards stranger vs. familiar conspecific (impaired social novelty)	21 days	Single chamber dimensions, length, width, height (all 3 chambers are identical): Rat: 40 cm × 80 cm × 40 cm. Mouse: 20 cm × 40 cm × 20 cm Object/stranger enclosure dimensions, diameter × height (both enclosures are identical): Rat: 15 cm × 25 cm; Mouse: 7 cm × 15 cm. Bar spacing 7–10 mm Conspecific and test animals - strain, age and sex matched Habituation: 3–10 min; Sociability phase: 3–10 min; Social novelty phase- 3–10 min Conspecific and test animals - strain, age and sex matched
Impaired communication	Social transmission of food preference (STFP)	Diminished preference towards cued food vs. non-cued food	21 days	Pre-test fasting 12 hrs. Flavors 1% Cinnamon and 2% Cocoa (weight/weight) Demonstrator food exposure 60 min Demonstrator-observer interaction 30–60 min Test duration 3–5 min Frequency resolution 488 Hz Time resolution 1 ms Frequency band 150–180 kHz
Restricted, repetitive and ritualistic behaviors	Ultrasonic vocalization in adults and in pups (USV-A; USV-P)	Pups: reduced number and/or impaired structure of ultrasonic calls upon the separation from the dam	1 day	Conspecific and test animals – strain and age matched Test duration 3–5 min Frequency resolution 488 Hz Time resolution 1 ms Frequency band 20–100 kHz Test duration 10–20 min Habituation – ½ of the test duration Number of objects – 20 Object diameter – 1.5 cm
Behavioral rigidity	Self-grooming (SG) Object burying (OB) Reversal learning in T-maze (RLTM)	Adults: reduced number and/or impaired structure of ultrasonic calls emitted by the test animal upon the introduction of the intruder into the home cage Increased number of episodes and/or of total duration of self-grooming Increased number of buried unfamiliar objects Increased number of trials required for switching to the new location of the food reinforcer; increased number of errors during the reversal phase vs. the number of errors during the acquisition phase	28 days 15 days 66 days 18 days	Single arm dimensions, length × width × wall height. Rat 50 cm × 10 cm × 20 cm; mouse 30 cm × 7 cm × 10 cm Food restriction- target weight 85% of baseline Training: 2–4 days; pass threshold 4–6 completed trials in 15 min. Acquisition and reversal learning criterion: 6 consecutive correct responses. See MWM in Table 1
	Morris water maze (MWM)	Inability to learn new platform location (reversal and double-reversal paradigm)	21 days	

*Analysis and interpretation.* An exacerbated increase in body temperature is an indicator of anxiety. The increase can be expressed either in absolute numbers, or as a percentage of the averaged baseline value.

#### *Novelty-suppressed feeding*<sup>52</sup>

[File name: 3 Anxiety-NSF CRF; 3 Anxiety-NSF CDE Chart]

*Rationale.* Novelty-suppressed feeding is a conflict-based test, in which a fasted animal faces a choice between consuming food in a novel stressful environment and staying away from food in a presumably safe location.

*Procedure.* The test animal is food-deprived in their home cages for 24 h. The arena can be an open field, a novel home cage, or any comparable large container. The food platform can be a Petri dish, or any accessible container, which contains a food sample (e.g., standard food pellet), placed in the center of the arena. The food platform is illuminated brighter than the periphery (e.g. 150–200 Lx vs. 20–40 Lx). The animal is placed in a corner of the arena and is allowed to explore for 5–10 min. After the test, the animal is returned in the home cage, which now contains standard diet.

*Analysis and interpretation.* The measure of anxiety is the latency to consume food in the novel environment. If the animal fails to consume the food, the total test duration is assigned. As a supplementary index, the latency and number of approaches to the food without consuming it can also be measured; increased number of such aborted attempts is an additional measure of anxiety. At the same time, the animals should consume food on return to their home cage within 1 min. Failure, or increased latency of food consumption in the home cage, may suggest anhedonia rather than anxiety. Even though the animals are fasted prior to the test, depending on the type of food sample, the motivation between exploration and consumption may be different. If the food is novel (e.g., not a standard food pellet), it is advisable to familiarize the animal with this type of food prior to the test.

#### *Panic-like behavior induced by stimulation of DPAG*<sup>53,54</sup>

[File name: 5 Anxiety-PA CRF; 5 Anxiety-PA CDE Chart]

*Rationale.* Panic behavior is mediated by dorsal periaqueductal gray matter (DPAG). In rodents, panic-like behavioral responses can be elicited by incremental electrical stimulation applied to DPAG; quantification is based on determining stimulation thresholds for inducing typical behavioral reactions.

*Procedure.* The test animal is implanted with chronic stimulating electrode into DPAG (typically lateral part). Electrical stimulation is performed in the freely moving animal after a 1-week recovery. Typical parameters are 30 s trains, 60 Hz, starting with 5  $\mu$ A, with 5  $\mu$ A increments, applied 5 min apart. Behavior is analyzed during the stimulations using an ordinal scale as follows: (1) Exophthalmos; (2) Immobility,

behavioral arrest accompanied by the increase of muscle tone in the neck and limbs; (3) Trotting, fast locomotion with out-of-phase stance and swing movements of contralateral limbs and the elevation of trunk and tail; (4) Galloping, running alternating stance and swing movements of anterior and posterior limb pairs; (5) Jumping, upward leaps; and (6) Defecation and micturition, ejection of feces and urine. Many of the described behaviors may be manifestations of seizures (e.g., (1) and (2), nonconvulsive seizures; (3), (4), and (5), “running” seizures). It is recommended that EEG is recorded during and shortly after DPAG stimulation so that panic-like reactions can be discerned from seizures.

*Analysis and interpretation.* Minimal current required to induce each of the described behavioral reactions is determined. Panic behavior is characterized by a decrease in the threshold for each behavior. It also should be noted that DPAG is only one component of a network that produces panic-like behavior; the network includes most of the mid-brain tectum.<sup>55</sup>

## AUTISM

### J. Veliskova, A.S. Galanopoulou, A. Mazarati

#### *Three-chamber test*<sup>56,57</sup>

[File name: 1 Autism-3CH CRF; 1 Autism- 3CH CDE Chart]

*Rationale.* Deficits in social interaction are a core symptom of autism. The three-chamber test measures a rodent’s motivation to engage and interact with conspecifics (Table 5).

*Procedure.* The apparatus is a chamber divided in 3 equal-sized, connected compartments. The central (starting) compartment is empty. The terminal compartments contain identical enclosures (e.g., cylindrical with vertical bars). Typically, the test animal is assessed during 3 sessions of similar duration, the first of which is habituation, during which only the test animal is placed in the apparatus. Two consecutive phases follow, which assess sociability and social novelty. During the sociability phase, one of the enclosures contains a conspecific unfamiliar to the test animal and the other contains an inanimate object. For the social novelty phase, the conspecific (now familiar) remains in position, while the object is replaced with another conspecific unfamiliar to the test animal (thus the test animal encounters one familiar and one novel conspecific). During each phase, the animal freely explores the apparatus. In pups, a modified version has been used,<sup>57</sup> which includes shorter session periods (e.g., 3 min), and 3 experimental conditions: habituation, exposure to a stranger, and exposure to a familiar (same litter) pup. Entries to each chamber, time spent in each chamber, and behaviors/interactions with other animals are recorded.

*Analysis and interpretation.* During the sociability phase, the exploration of the conspecific and the inanimate object

are recorded. Animals with sociability preferentially engage the conspecific; equal exploration of the conspecific and inanimate object is interpreted as lack of sociability. During the social novelty phase, normal animals preferentially engage with the novel, unfamiliar conspecific; again, lack of the preference suggests diminished sociability and social memory. The combination of the 2 phases increases the overall sensitivity of the test. Most commonly analyzed is total time engaging with the content of each enclosure (i.e., attempts of direct contact). The number of contacts can also be considered. Total time spent in each of the compartments is often calculated, although this may not be a particularly sensitive outcome: for example, autism-like behavior may include spending more time in the compartment with the conspecific/novel conspecific, but away from the enclosure, and engaging in displacement behaviors, such as grooming. Derivative indices are often used for statistical normalization. For example, sociability index is calculated as  $[T1/(T1 + T2)] \times 100 - 50$ , where T1 and T2 represent the time spent near the conspecific and inanimate object, respectively.

#### *Social transmission of food preference*<sup>58</sup>

[File name: 5 Autism-STFP CRF; 5 Autism-STFP CDE Chart]

**Rationale.** Deficits in social communication are a core symptom of autism. SFTP is used to examine the equivalent of non-verbal communication in rodents, which roughly parallels eye contact in humans.

**Procedure.** The test includes 2 groups of animals: demonstrators (naive subjects) and observers (test subjects). All animals are fasted for 12 h prior to the test. The test starts with cue acquisition, when the demonstrator consumes a flavored food (typically either 1% w/w cinnamon or 2% w/w cocoa is mixed with standard food), with the flavor serving as a cue. After 1 h spent with the cued food, cue transmission follows, whereby the demonstrators are brought in contact with the observers for 30 min. During the interaction, the observers pick up the cue. The test culminates in the cue recognition when the observer is given a choice between the cued and noncued food samples.

**Analysis and interpretation.** If during the cue transmission phase the observer engaged with the demonstrator, then during the cue recognition, the observer would preferentially consume the cued food (i.e., of the same flavor that the demonstrator consumed during cue acquisition). If the interaction between the demonstrator and the observer was lacking, or truncated, and the latter did not acquire the cue, it consumes equal amounts of cued and un-cued food. Therefore, social interaction is inferred by calculating the ratio of the consumed cued to noncued food. Reference memory impairments can affect performance in this task, which appears to depend on the integrity of the CA2 region of the hippocampus. Other tests should be used to dissociate the memory component from social abilities.

#### *Ultrasonic vocalizations—pups*<sup>59</sup>

[File name: 7 Autism-USV-P CRF; 7 Autism-USV-P CDE Chart]

**Rationale.** The test examines the equivalent of verbal communication. Rodents communicate with each other through ultrasonic calls. When applied in pups, the test exploits the observation that pups emit ultrasonic calls to the dam upon separation from the latter.

**Procedure.** The major equipment is an ultrasonic microphone placed in a recording chamber insulated from environmental noise and connected via a data acquisition board to a computer with an acquisition/analysis software. Pups are separated from the dam one at a time by placing them in a sound-proof chamber for 3–5 min, during which ultrasonic calls are recorded.

**Analysis and interpretation.** Deficits in communication are evident by a reduced number of overall ultrasonic calls, or impaired call structure, which involves analysis of calls by waveform types (e.g., upward, downward, 2-syllable, and chevron).

#### *Ultrasonic vocalizations—adults*<sup>60,61</sup>

[File name: 6 Autism-USV-A CRF; 6 Autism-USV-A CDE Chart]

**Rationale.** The rationale is the same as described earlier, but the premise is different in that adult rodents emit ultrasonic calls as means to communicate with each other upon an encounter.

**Procedure.** The test includes 2 subjects: the resident, which is the test animal, and the intruder. The animals are age-matched but can be of either the same or the opposite sex, depending on the test aims. The test is performed in the resident's home cage, which is equipped with the ultrasonic microphone; the intruder is placed with the resident for 3–5 min, during which ultrasonic calls are recorded. During the test, the intruder is generally silent, so that the majority of the calls are emitted by the resident.

**Analysis and interpretation** is similar to the one applied to pups.

#### *Self-grooming*<sup>58,60</sup>

[File name: 4 Autism-SG CRF; 4 Autism-SG CDE Chart]

**Rationale.** Repetitive, restricted, and ritualistic behaviors are symptoms of autism. Generally, grooming is a normal part of the rodent behavioral repertoire. Exacerbated self-grooming is interpreted as a manifestation of repetitive ritualistic behavior.

**Procedure.** The test typically lasts 10–20 min. The test animal is placed inside a cylinder with transparent walls for 10–20 min. After a habituation period (one-half of the total test duration) grooming is recorded.

Table 6. Attention deficit/hyperactivity disorder

Examined impairment	Test	Main indicator of the deficit	Youngest reported age	Examples of main settings
Impulsivity and Attention deficit	5-choice serial reaction time task (5-CSRT) Lateralized reaction time task (LRTT)	Impulsivity: increased number of incorrect/premature responses. Attention deficit: increased number of omissions	45 days	Both tests: target weight: 85% of initial weight 5-CSRT: Single session duration 30 min; number of trials per session < 100; Stimulus duration 0.125, 0.25, or 0.5 s; Inter-training interval – 0.5–4.5, 5, 9, s. LRTT: Variable target stimulus duration condition-duration - <60 min. Variable target stimulus duration condition- trial number - <160. Each test lasts several weeks (depending on the training curve).
Attention deficit	Attentional set-shifting task (ASST) <sup>a</sup>	Delayed, or lack of the ability to learn a new set of environmental cues (dimension) that contain food reinforcer.	26 days	Target weight: 85% of initial weight Digging media scent examples: cinnamon, cocoa, cloves Digging media texture examples: sawdust, sand, plastic Bowl texture examples: sandpaper, cloth, wax paper Success criterion: 6 consecutive correct responses Test duration 3 days.

<sup>a</sup>No literature records were found on applying this, or similar tests in association with epilepsy models.

*Analysis and data interpretation.* Increased grooming as compared with normal animals indicates the presence of ritualistic behavior. Alternatively, increased grooming can be a manifestation of anxiety, particularly in the novel environment; therefore, the behavior should be evaluated in the context of other relevant impairments. The behavior is quantified by calculating cumulative grooming time and the number of grooming episodes.

#### Object burying<sup>60,62</sup>

[File name: 2 Autism-OB CRF; 2 Autism-OB CDE Chart]

*Rationale.* This test also examines repetitive, restricted behavior. Normal rodents exhibit burying behavior; however, exacerbated burying may be indicative of perseverative behavior.

*Procedure.* The exam lasts 10 min. The test animal is placed in a cage with bedding for 10 min for habituation and removed. Objects, typically marbles 1.5 cm in diameter, are laid on top of the bedding, equidistant from each other in a 4 × 5 arrangement. The animal is returned to the test cage for 10 min, and then removed. The number of marbles fully covered by the bedding is recorded.

*Analysis and interpretation.* Normal animals bury approximately 50% of marbles. Increased burying activity is interpreted as perseverative, repetitive behavior, and is evident as a larger number of buried marbles. It has been suggested that the test is more specific toward the repetitive behavior than anxiety<sup>62</sup>; it thus can complement, or replace, the self-grooming test.

#### Reversal learning in T-maze<sup>63</sup>

[File name: 3 Autism-RLTM CRF; 3 Autism-RLTM CDE Chart]

*Rationale.* Behavioral rigidity/insistence on sameness is an autistic trait. Reversal learning in the T-maze examines the ability of an animal to adapt to changes in environmental cues that lead to the reward.

*Procedure.* Two major paradigms can be used: deterministic and probabilistic.

*Deterministic paradigm:* The test requires food restriction with a target weight of 85% of baseline. The test is performed in a standard T-maze. It begins with 5 days of habituation, when the test animal is placed in the apparatus with cups containing food pellets located in the opposite ends of the arms. The training phase consists of 10 training trials per day. One arm of the T-maze is designated as the correct arm, where a reinforcer (e.g., sugar cube or cereal) is placed at the end of the arm. The animal is placed at the starting arm of the T-maze stem and is given a choice of entering either arm. If the animal chooses the correct arm, it is given time to consume the food and then guided back into the start

arm for the next trial. For each successive trial, the reinforcer is always placed in the same arm. The criterion for the task acquisition is an average of 80% correct responses across 3 days of testing. Once the criterion is reached, the reversal phase is performed. The reversal phase consists of switching the reinforcer location to the opposite arm. The procedure follows the protocol described for training.

The probabilistic paradigm is different from the deterministic paradigm in that instead of placing the food consistently into one of the arms, it is placed in the “correct” arm in 8 of 10 trials, and in the remaining 2 of 10 trials the food is present in the “incorrect” arm (for the reversal learning, the 8:2 ratio is switched between the 2 arms). This paradigm allows determining more subtle impairments which may not be detectable under the deterministic paradigm.

For testing in pups, see Ref. 64.

*Analysis and interpretation.* The following parameters are analyzed for both acquisition and reversal phases. (1) Trials to criterion (i.e., the animal visits the correct arm on average 80% of trials); number of perseverative errors (i.e., the inability to inhibit the previously relevant choice pattern), and number of regressive errors (i.e., the ability to maintain the new choice pattern after being initially selected). In normal animals, the number of trials to reach the criterion and the number of errors upon reaching the criterion are similar between the acquisition and reversal phases. Increased number of trials to reach the criterion and/or larger number of errors during the reversal phase is regarded as an indicator of behavioral rigidity. It should be mentioned that although the test is relevant to screen for autism-like phenotypes, it is similarly relevant for impaired frontal cortex and basal ganglia function, which may occur for reasons other than autism.

#### Morris water maze

[File name: 5 Memory-MWM CRF; 5 Memory-MWM CDE Chart]

*Rationale, analysis and, interpretation.* This is similar to the reversal learning. In the reversal and double-reversal paradigm of the Morris water maze, perseverative behaviors are evident if an animal keeps attending to the old location of the platform.

## ATTENTION DEFICIT/HYPERACTIVITY DISORDER

### P-P Lenck-Santini, A. Mazarati

5-Choice serial reaction-time task(5-CSRTT)<sup>65,66</sup>

[File name: 1 ADHD-5CSRT CRF; 1 ADHD-5CSRT CDE Chart]

*Rationale.* This test assesses visuospatial attention and impulsivity. It is performed in an operant chamber equipped with 5 apertures and a food dispenser (Table 6). The 5-CSRTT requires an animal to correctly identify which of the 5 apertures has been briefly illuminated, via a nose poke; a correct response is rewarded by delivering food through the food dispenser. The difficulty of the task is controlled by the length of time the aperture is illuminated: shorter illumination time requires the animal to pay greater attention. Between the trials, there is a short interval during which the animal is expected to withhold the response—to exercise an inhibitory control. Hence, the test affords gauging both attention deficit and impulsivity.

*Procedure.* Prior to and during the test, the test animal is food restricted until it reaches 85% of the baseline weight. Prior to training, the animal is allowed to habituate in the operant chamber and to learn that the food magazine dispenses food pellets upon nose-poking a hole. The training consists of sequentially switching each of the lights on and off, and the animal gradually learning that food is dispensed only if it pokes the hole under the correspondent light (i.e., the correct hole), and only after the light is switched on and off (i.e., at the correct time). Poking an incorrect hole points toward lack of attention; prematurely poking a correct hole points toward hyperimpulsivity. During each session, multiple trials are performed. The duration of the intertrial interval and the duration of the stimulus presentation can be varied. Shortening intervals between stimuli increases attention errors. Increasing intertrial intervals increases a chance of premature responses. Therefore, by varying the intertrial intervals, more reliable information can be obtained about hyperimpulsivity.

*Analysis and interpretation.* Several types of errors can be measured: (1) Attention errors—visits to the wrong hole.

**Table 7. Example of settings for the Attentional set shifting task**

Task	Dimensions
Simple discrimination (SD)	<b>Odor 1<sup>a</sup></b> vs. Odor 2
Compound discrimination (CD)	<b>Odor 1/Wall texture 1</b> vs. Odor 2/Wall texture 2
CD reversal	<b>Odor 2/Wall texture 1</b> vs. Odor 1/Wall texture 2
Intradimensional shift (IDS)	<b>Odor 3/Wall texture 1</b> vs. Odor 4/Wall texture 2
IDS reversal	<b>Odor 4/Wall texture 1/Odor3/Wall texture 2</b>
Extradimensional shift (EDS)	<b>Bedding texture 1/odor 5</b> vs. Bedding texture 2/odor 6
EDS reversal	<b>Bedding texture 2/odor 5</b> vs. Bedding texture 1/odor 6

<sup>a</sup>Relevant stimulus for each task is indicated in bold.

Table 8. Psychosis

Examined impairment	Test	Main indicator of the deficit	Youngest reported age	Examples of main settings
Impaired sensorimotor gating	Acoustic startle response with habituation (ASR)	The amplitude of motor startle response does not decrease upon the repeated presentation of auditory stimuli	10 days	Chamber ambient noise 70 dB; Chamber habituation 5 min; startle pulse intensity 90, 105, 120 dB; startle pulse duration 40 ms; inter-trial interval random, 15 s average; test duration ≈ 60 min.
	Prepulse inhibition (PPI)	Weak auditory stimulus (i.e. prepulse) does not inhibit motor startle response to the subsequent strong auditory stimulus (i.e. pulse)	10 days	Chamber habituation 5 min; startle pulse intensity 120 dB startle pulse duration 40 ms; prepulse intensity 2, 4, 8, 16 dB; prepulse duration 20 ms; delay between prepulse and startle pulse 100 ms; inter-trial interval random, 15 s, average; test duration ≈ 60 s
Dysfunctional Dopaminergic and/or glutamatergic transmission	Psycho-stimulant-induced locomotion (PSL) <sup>a</sup>	Increased motor activity in response to amphetamine, MK801, ketamine. Increased climbing behavior in response to apomorphine		Drug doses, mg/kg, i.p. or s.c.: Apomorphine 0.75; amphetamine 1.5; MK-801 0.16; Ketamine 5. Observation period ≤30 min

<sup>a</sup>No literature records were found on applying this, or similar tests in association with epilepsy models.

Increased number of visits to a wrong hole is evidence of attention deficit; (2) Omissions --failures to respond within 5 s after the light is off. Increased number of omissions also suggests lack of attention; (3) Premature responses—when the animal reacts before the stimulus sequence is completed. Increased number of premature responses is evidence of hyperimpulsivity.

#### Lateralized reaction time task(LRTT)<sup>38,67</sup>

[File name: 3 ADHD-LRTT CRF; 3 ADHD-LRTT CDE Chart]

*Rationale* is similar to 5-CSRTT. During the LRTT, the test animal engages in a variable-duration fixation response while waiting for the delivery of a visual target (light) in the left or right visual field. During this fixation response, the animal must divide and orient their attention, monitoring both locations for the stimulus delivery; because the temporal onset and duration and spatial localization of the target are unknown, the subject will “miss” the target if they attend to only one location and/or fail to sustain their attention.

*Analysis and interpretation.* Dependent measures include the following: (1) discriminative response accuracy (i.e., correct responses/[correct + incorrect responses])—measure of attention; (2) omission rate (as a percent of total trials)—measure of attention; (3) total anticipatory responses—measure of impulsivity; (4) mean initiation latency/trial (i.e., the average interval between illumination of the center nose poke aperture and the initiation of the observing response)—measure of impulsivity.

#### Attentional set shifting task<sup>68,69</sup>

[File name: 2 ADHD-ASST CRF; 2 ADHD-ASST CDE Chart]

*Rationale.* The test examines the ability of an animal to form attention sets relevant for successful retrieval of positive reinforcer (food). The reinforcer is consecutively placed in environments characterized by different dimensions (see below), and the ability to adapt to new conditions associated with the reinforcer is quantified. The test is primarily used to gauge attention but requires a degree of learning flexibility. In contrast to 5-CSRTT and LRTT, the task does not require special equipment, and can be completed within several days instead of weeks. However, the test does not allow the examination of impulsivity.

*Procedure.* The apparatus is a cage (e.g., 40 × 70 × 20 cm), with a plastic divider in the middle containing a sliding door at the bottom, a set of digging bowls filled with digging media, and standard food bait. The bowls differ in dimensions; typically 3 dimensions are used, with 3 characteristics for each. (1) Scent of digging media (e.g., cinnamon, cocoa, cloves); (2) Texture of digging media (e.g., sawdust, sand, plastic); (3) texture of the

**Table 9. Aggression**

Examined impairment	Test	Main indicator of the deficit	Youngest reported age	Examples of main settings
Exacerbated aggressive behavior	Resident-intruder test (RIT)	Shortened latency to, and increased frequency of aggressive behaviors	50+ days	Only used in males. Number of trials $\approx 10$ . Test duration $\leq 10$ min
Excessive social dominance	Tube dominance test (TDT)	Increased number of displacements of the conspecific	50+ days	<p>Tube dimensions.</p> <p>Rats: length 48 cm; diameter 5.5 cm; entry to door: 20 cm; distance between doors 8 cm.</p> <p>Mice: length 30 cm; diameter 3.5 cm; entry to door: 13 cm; distance between doors 4 cm. Number of trials <math>\approx 10</math>. Test duration <math>\leq 10</math> min</p>

bowl (e.g., sandpaper, cloth, wax paper; Table 7). The goal is to train the test animal that the food is associated with a certain dimension (i.e., bowl texture + digging media texture + digging media scent).

After initial habituation, the animal is given a series of discriminations using 3 different pairs of stimulus exemplars of each dimension: simple discrimination (SD); compound discrimination (CD); CD reversal; intradimensional shift (IDS); IDS reversal; extradimensional shift (EDS); EDS reversal (Table 7).

For test in pups, see Ref. 70.

*Analysis and interpretation.* For each stage of the test, the number of errors or trials to reach criterion is calculated. The criterion is typically set as 6 consecutive retrievals of the reinforcer. Higher numbers of either errors or trials to the criterion point toward attention deficit. In addition, the EDS/IDS ratio is calculated. Normally, IDS is faster than EDS; failure to form relevant attentional set is characterized by the similar rates of IDS and EDS.

## PSYCHOSIS

L.E. Kalynchuk, N.C. Jones, A. Mazarati

*Acoustic startle response*<sup>71,72</sup>

[File name: 1 Psychosis-ASR CRF; 1 Psychosis-ASR CDE Chart]

*Rationale.* Habituation to a repeated startling stimulus is one of the oldest recognized forms of learning (Table 8). The whole-body response to the startle can also be used as a measure of anxiety. Both of these outcomes have relevance for psychosis/schizophrenia phenotypes.

*Procedure.* The test animal is placed in the test chamber—a standard commercially available startle box—and exposed to auditory stimuli. The chambers measure the amplitude of body movement triggered by the stimuli. For assessment of the acoustic startle response, 3 stimulus intensities of 40 msec duration are typically used (such as 90, 105, and 120 dB). The stimuli are presented in a quasi-random order so that an equal number of presentations of each stimulus intensity is included, and no single intensity is presented more than twice in succession. At least 10 trials at each stimulus intensity should be used to obtain reliable results. For startle habituation, a single stimulus intensity is repeatedly presented throughout the session using either a fixed or variable interval. Responses normally decline (habituate) over trials.

For testing in pups, see Ref. 73.

*Analysis and interpretation.* Greater startle amplitude to a given stimulus is indicative of elevated anxiety. For habituation of startle, the amplitude of startle responses diminishes in normal animals upon repeated presentation of auditory stimulus, but if this does not occur, habituation deficits are apparent.

*Prepulse inhibition*<sup>71,72</sup>

[File name: 2 Psychosis-PPI CRF; 2 Psychosis-PPI CDE chart]

**Rationale.** Sensorimotor gating is the ability of the central nervous system (CNS) to adapt to sensory stimuli upon their repeated presentation. Schizophrenia, a disorder often associated with psychoses, is characterized by impairments in sensorimotor gating that is an inability to adapt to such repeated stimuli. For the examination of sensorimotor gating, auditory stimulation is commonly used.

**Procedure.** The same startle boxes are used for this test as for startle response assessment. In this test, the attenuation produced by a low intensity stimulus presented just before the startle stimulus is assessed. Prepulse inhibition (PPI) is typically assessed during the same session as the startle response test. The test animal is exposed to 6 different types of acoustic stimuli in a randomized order: pulse alone (120-dB noise for 40 msec), no stimulus (no stimulus is presented), and 4 separate prepulse + pulse combinations, with prepulse set at 4 sound levels of 2, 4, 8, and 16 dB above background for 20 msec followed 100 msec later by a 40-msec pulse at 120 dB. A total number of 12 trials under each acoustic stimulus condition are presented with an intertrial interval ranging from 5 s to 30 s. The inclusion of 4 pulse-alone trials in the beginning of the experiments normalizes the response of the animal, as there is rapid habituation to the startle responses seen within the first few trials.

For testing in pups, see Ref. 73.

**Analysis and interpretation.** The presentation of a prepulse reduces the amplitude of startle response upon presentation of pulse stimulus in normal animals. Lack of inhibition of startle response in the prepulse-pulse sequence points to psychosis-like sensorimotor gating impairments. It should be considered, however, that the disruption of PPI is relevant, but not specific for schizophrenia, as it occurs in a variety of neuropsychiatric and neurologic conditions.

*Psychostimulant-induced locomotion*<sup>74</sup>

[File name: 3 Psychosis-PSL CRF; 3 Psychosis-PSL CDE Chart]

**Rationale.** Schizophrenia is characterized by the dysfunction of dopaminergic and glutamatergic neurotransmission, which is manifested as an exacerbated response to psychostimulants.

**Procedure.** For the psychostimulant locomotion test, the test animal is placed individually into an open arena and baseline spontaneous activity is recorded for 30 min. Animals are then injected with a pharmacologic agent (such as d-amphetamine, ketamine, MK-801) and replaced in the arena, after which locomotor activity is recorded and calculated for the following ~90 min. For the climbing test, the animal is injected with apomorphine and placed inside a tall, meshed cylinder. Climbing

is scored every ~2 min using an ordinal scale: 0, all 4 paws on the floor; 1, gripping vertical bars with forepaws; 2, gripping vertical bars with 4 paws.

**Interpretation.** Psychosis-like behavior is characterized by increased drug-induced locomotor activity, and/or increased climbing score in the apomorphine test.

**AGGRESSION****N.C. Jones***Resident-intruder test*<sup>75</sup>

[File name: 1 Aggression-RIT CRF; 1 Aggression-RIT CDE Chart]

**Rationale.** Male rodents are territorial and will defend their environment against unfamiliar male intruders (Table 9).

**Procedure.** The apparatus is a cage with a floor space of about 0.5 square meters. Prior to the test proper, the test subjects (residents) are housed in the apparatus with ovariectomized females for at least 1 week, to establish territoriality. During this period, the apparatus is not cleaned. The test proper is best performed during the dark phase of the light-dark cycle. For the test, female is removed from the apparatus, and an unfamiliar male (the intruder) is introduced. The intruder should be slightly smaller than the resident. The animals are allowed to interact for 10 min. Up to 2 trials can be performed in one day, each with different intruders.

**Analysis and interpretation.** Several interaction behaviors occur, some of them being aggressive acts (rearing; lateral threat; upright posture; clinch attack; chasing; keeping down; bite attacks). Defensive behaviors may also be present (submissive posture; moving away; flight; freezing; defensive upright posture). Other neutral interaction and non-interaction behaviors are also recorded (Table 9). For each type of behavior, the latency, number of episodes, and duration are recorded. Aggression can be expressed by calculation the proportion of aggressive behaviors.

*Tube dominance test*<sup>76</sup>

[File name: 2 Aggression-TDT CRF; 2 Aggression-TDT CDE Chart]

**Rationale.** The test is used to study aggression by gauging social dominance.

**Procedure.** The apparatus is an acrylic glass tube with a guillotine dividing door in the middle and attached to holding boxes on each side. Tube diameter is such that it fits only one animal. Prior to and throughout the test, the animals are on food restriction, with the goal weight 85% of the baseline. When the goal weight is reached, the animals are trained. A food sample is placed in one holding

box, and the animal placed in the opposite holding box. The dividing door is opened and the animal is allowed to reach the opposite holding box via the tube, and to consume the food. Training continues for 3 consecutive days, with 2–3 daily sessions. For the test proper, 2 animals are placed in the opposing holding boxes, and the dividing door is open. The animals both then proceed through the tube to the opposite holding box, only to encounter each other in the tube. This creates a conflict situation, whereby the dominant animal starts pushing the recessive animal back until the point that the latter is pushed into the holding box entirely. At this point, the dominant animal is declared a winner, and the trial ends. The test procedure is repeated for 5 consecutive days with typically 3 trials per day, so that the subject is tested against 15 different opponents.

*Analysis and interpretation.* Social dominance is evaluated as a proportion of wins. Increased number of wins, as measured versus normal conspecifics, is an indicator of higher level of aggression. At the same time, decreased number of wins can be an indicator of social anxiety.

## DEVELOPMENTAL MILESTONES

A.S. Galanopoulou, A. Nehlig

[File name: *Developmental milestones CRF; Developmental Milestones CDE Chart*]

*Rationale:* To assess the effects of seizures, epilepsy, and their comorbidities on the development of immature rodents with, or at risk for these conditions, a minimal daily battery assessing neurodevelopmental growth can be utilized to assess growth, motoric function, and coordination. It requires  $\leq 5$  min per pup per day. The proposed tests are as listed below. Species/strain differences in time of maturation exist and reported maturation time refers to Sprague-Dawley rats.

*Procedures:* Various simple procedures are conducted to assess developmental progress. These should be assessed daily at least until the milestone is reached, or at specific developmental periods that coincide with the expression of a specific phenotype (seizures, comorbidity) so as to evaluate for possible regression or new developmental deficits. Many of the outcomes can be expressed as binary (e.g., failures vs. successes; present vs. absent) or analyzed as parametric values derived from the successful trials. We list these in no preference of importance.

### Physical Milestones

*Weights.* Daily weights can give an idea about failing to thrive or poor body growth, or conversely a tendency to obesity or increased body growth. If a difference is observed, body lengths may be useful to differentiate.

*Pinna detachment and ear development.* Rat pups are born with pinna (earflaps) attached to the skull. Pinna detachment from the skull occurs between postnatal day (PND)1-7 in Sprague-Dawley rats and, in general, around PND2-3.<sup>77</sup> The day when each pinna detaches can be recorded. Ear canals are closed at birth. Opening can be documented by an experienced observer or by using otoscope and occurs by PND7-10.<sup>78</sup>

*Body fur.* Rat pups are born without fur; fur appears by PND7-10.<sup>78</sup>

*Eye opening day.*<sup>57</sup> Day when eyes first open. It varies with species/strains. In Sprague-Dawley rats, eye opening occurs between PND13 and 15. The day when each eye opens can be recorded.

*Teeth eruption.* In albino rats, mandibular and maxillary incisors first erupt from the rat's gums around PND8-10.<sup>79</sup> Each rat has 3 sets of molars (12 in total): the first molars erupt around PND19, the second around PND21, at which age rats can be weaned, whereas the third occurs around PND35-40.<sup>79</sup> Different metrics to indicate the day of teeth eruption, for example, tooth was 0.5 mm, and the parameters each investigator considers should be included in the manuscript.

### Reflex Ontogeny and Motor Behaviors

*Surface-righting reflex.*<sup>57</sup> Pups are placed in the supine position and the time taken to acquire the prone position, standing on 4 limbs, is measured. The test is terminated at 60 s (failure score); the mean time for a successful trial is usually a few seconds at PND4. This reflex usually matures by PND9 in Sprague-Dawley rats. Single measurements are routinely done at PND4 and 5 for easy discrimination.<sup>57,80,81</sup>

*Air righting reflex.* Pups are held upside down from a height less than a meter from a cushion and are released. Normally, pups right themselves so that they land on their 4 paws (acceleratory placing response). In rats, air-righting reflex appears around PND6-7 and is completed around PND14-16.<sup>82</sup>

*Open field activity.*<sup>57</sup> Rat pups are placed with forelimbs at the center of a 12.5 cm circle, and the time taken to escape from this circle with both forelimbs placed outside is measured. The test can be terminated at 60 s (failure score). There is a steady improvement of this score between PND4 and 20 in Sprague-Dawley rats. Adjustments of the open field size and termination point can be done for smaller-size mouse pups, after optimization of conditions.

*Negative geotaxis.*<sup>57</sup> Pups are placed snout downward on a tilted surface, and the time taken for the pup to turn 90 degrees and start climbing upward is noted. The test is terminated at 60 s (failure score). Angle of tilted surface can vary but should be within 20 to 45 degrees, but should be kept steady within the same study to permit comparisons. It usually matures by PND14 in Sprague-Dawley rats. Its measurement at PND9 has been performed routinely, and at that

age, it should not take longer than 30 s for the pup to complete the trial.<sup>80,81</sup>

**Horizontal bar.**<sup>83</sup> Pups are placed with limbs grasping a horizontal bar (1.5–2 cm diameter for rats, 25 cm long) placed on an elevated level (i.e., 30 cm high from a bedded surface) and the time taken for the pup to fall off is recorded. For mice, thinner bar diameters are being utilized (2–6 mm). The test is terminated at 60–120 s or at the time-point when a pup loses grasp of the bar (failure). Usually this test is tested after eye opening, for example, starting on PND13 in Sprague-Dawley rats.

**Rooting reflex.** Bilateral touching of pup's snout induces forward motion and rooting of its head. Response: present/absent. It usually develops by PND3.

**Vibrissae placing.** The pup is suspended by its tail and the response to a pencil touching the vibrissae is noted: normally the head should rise with forelimbs trying to grasp the pencil. Response: present/absent. It usually develops by PND8.

**Forelimb placing.** The pup grasps a dowel being stroked against the paw. Response: present/absent.

**Pattern of ambulation and gait.** Pups are able to crawl with forelimbs mostly by PND8 and able to walk by PND14, although hindlimbs may drag; rearing is noted by PND18. Tendency to circle or limp is worth noting. Gait assessment in ambulatory rats can be visual or automated. Descriptors can be used under "other gait" to describe whether gait is circular, zigzag, ataxic, or hemiparetic with falls on one side, if abdomen is drag/parallel-high above ground when walking or describe the patterns of paw, stepping, or toe clearance movements as well as trunk position (side/mid) and stability (absent/present).<sup>84</sup> The reader may refer to the specific gait/locomotion scales used for specific neurologic models. The scale used can be logged under Gait/Scale. A tentative scoring system with 0 = normal and 3 = incapacitated is given as an example in the CRF.

**Foot-fault test.** The pups are placed on a horizontal grid (50 × 40 cm, square size 3 × 3 cm, wire diameter 0.4 cm). The number of forelimb or hindlimb slips through the grid as well as right-left differences are noted. Each session can last 180 s.

**Modified grip-traction test.** The pup is hung onto a 0.6 cm horizontal rope by its forepaws. The time until the pup falls off onto a bedded surface is recorded. The maximum session time can be set to 60–180 s, according to age. The average duration for a successful trial is 10 s at PND12.<sup>80,81</sup> This test is most commonly performed early before eyelid opening to control for muscle strength and physical resistance and to eliminate any participation of emotivity or fear. However, the test can also be performed at older ages, if required by the experimental design.<sup>85</sup>

**Motor coordination.** This test adapted from Ref. 86 is usually performed around weaning (i.e., at PND21) and is divided in 3 phases. During the first phase, the animal is

forced to swim in a round container (15 cm diameter and 23 cm height) half full of water. The animal is swimming until finding by accident a metal rod (8 mm diameter) located deep inside the water. During the second phase, the animal has to climb along the rod (35 cm) to escape from the water. During the third phase, the rat has to reach and land onto a horizontal platform located at the top of the metal rod on which it can restore its normal quadruped posture. This test measures motor coordination, and the total time and time necessary for the animal to complete each phase can be recorded.

**Postural reflex.** The rat is lifted by its tail ~50 cm above the table. Normal response (score 0) is logged when both forelimbs extend toward the table. A score 1 is logged when one forelimb flexes and may indicate contralateral brain lesion, for example, postischemic.<sup>87</sup> Then the rat is placed on a table covered by a soft plastic-coated bench pad. With the tail held by examiner, gentle lateral force is applied behind the shoulders. If a rat gives reduced resistance and slides over one side reproducibly, a score 2 is logged and typically indicates brain lesion contralateral to the weak side.<sup>87</sup> The test is usually performed in older, ambulatory rats. The postural reflex test is a derivative of the Bederson scale<sup>87</sup> that was proposed as a crude neurologic assessment in the stroke field and also includes a score 3 if circling occurs. A variety of neuroscore systems have been developed for stroke models,<sup>87–89</sup> traumatic brain injury,<sup>90</sup> or other neurologic conditions that are beyond the scope of this manuscript.

**Hindlimb clasping.** The rat is lifted by its tail for 10 s. Normally hindlimbs are spread outward away from the abdomen. Hindlimb clasping is logged when hindlimb retraction occurs—partial or complete touching the abdomen—for more than 50% of the test time. Scoring can be qualitative (Yes/No/Unknown) or with predefined scale: 0 = normal, 1 = unilateral partial, 2 = bilateral partial, 3 = complete, touching abdomen.<sup>91,92</sup>

**Grooming.**<sup>57,93–95</sup> Grooming can naturally occur in pups. Increased number or duration of grooming behaviors can be observed in pathologic conditions, as early as the second week of life, and has been interpreted as stereotypies or autistic behavior, or may abnormal monoaminergic network activity.

## CONCLUDING REMARKS

### N.C. Jones

We provide here a narrative to support the use of preclinical CDEs developed for behavioral testing of animals in the context of epilepsy. Careful planning needs to go into specific paradigms for all research groups anticipating adoption of these CDEs. The list of the tests is far from exhaustive, and even for the described tests, many variations exist, with only selected protocols covered in this document. Still, we hope that these instructions may act as a good starting

reference for future studies, as well as for further refinement and expansion of a battery of assays to comprehensively examine and report neurobehavioral comorbidities of epilepsy in the laboratory setting. In addition, we anticipate that standardization of assessing behavioral outcomes, and concise reporting of methodologies will improve translational research of behavioral dysfunction in epilepsy models, as well as other disorders of behavior. Indeed, although we produced these documents primarily for research in epilepsy models, our preclinical CDEs represent the first available resource of its kind and could and should be utilized for all research groups studying neurobehavioral outcomes for the above-stated reasons.

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## AUTHORS CONTRIBUTIONS

AM and NCJ were responsible for compiling and editing the manuscript. The rest of the authors are listed in alphabetical order. All authors contributed to all portions of the manuscript to different extents; major contribution of each author is denoted under respective headings. LCHH was responsible for editing and standardizing CRF/CDE charts. JSSM was responsible for identifying appropriate ages for behavioral testing (Tables).

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Behavioral CDE and CRF files. The CDE and CRF modules linked to this article can be found and downloaded as a zip folder.