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Hybridizing behavioral models: A possible solution to some problems in neurophenotyping research?

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Abstract

The use of batteries of single-domain tests for neurophenotyping research is a common strategy to achieve higher data density and explore different behavioral domains. This approach, however, is accompanied by several methodological challenges, briefly discussed here. As an alternative, this paper advocates the wider use of extensive "hybrid" protocols that assess multiple domains in parallel, or logically/logistically combine experimental paradigms, in a way that disproportionately maximizes the number of tested phenotypes per experimental manipulation. Several examples of this approach are given in this paper, demonstrating the potential to reduce time, cost and subject requirements for the experiments. Offering behavioral analyses that are lacking in the standard single-domain tests, such "hybrid" models enable innovative modeling of neuropsychiatric disorders by more thorough and broader investigation of complex phenotypical characteristics.
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1. Introduction: current challenges

Although animal models are widely used for screening psychotropic drugs, testing neurobiological hypotheses and finding candidate genes for brain disorders (Bolivar et al., 2007; El Yacoubi and Vaugeois, 2007; Gould and Einat, 2007), neurophenotyping research is currently facing several challenges.

On one hand, mounting pressure due to increased animal/space costs (Lake et al., 1999) is leading to the extensive use of animals in intensive batteries to increase test information density (Godinho and Nolan, 2006; Sousa et al., 2006). Environment and prior test history may modify animal behavioral performance (Holmes and Rodgers, 2003; McIlwain et al., 2001), thereby influencing data validity and variability (Crabbe et al., 1999; Lathe, 2004; Wolfer et al., 2004). There are also growing concerns of neuroscientists for animal welfare (Warnick et al., 2006; Wurbel, 2007), and common problems with correct dissection of animal phenotypes in behavioral experiments (Cryan and Holmes, 2005; Kalueff et al., 2007d).

On the other hand, both academia and the industry need fast, low-cost, high-throughput behavioral screens for their expanding biomedical research (Crabbe and Morris, 2004; Godinho and Nolan, 2006; Tecott and Nestler, 2004). With the growing number of genetically modified animals (Hunter et al., 2000; MGI, 2007), including those with complex (Egashira et al., 2007; Hunter et al., 2000; Nolan, 2000) or overlapping (Clapcote et al., 2007; Szumlinski et al., 2005) phenotypes, the existing behavioral assessment techniques bolster this intensification in order to dissect multiple domains.

Moreover, it is becoming increasingly important to develop animal models for newly appreciated clinical phenomena (Kalueff et al., 2007d; Siegmund and Wotjak, 2007) and for integrative (Akiskal and Benazzi, 2005; Benazzi, 2006; Lara and Akiskal, 2006) vs. disorder-specific modeling of brain pathogenesis, see

Abbreviations: OCD, obsessive compulsive disorder; FST, forced swim test; MWM, Morris water maze; OFT, open field test; NT, neurological tests; ST, swim tests.

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(Einat, 2006; Einat, 2007; Gould and Einat, 2007) for discussion. Therefore, in addition to currently used experimental approaches (Tecott and Nestler, 2004) and "specific" animal models designed to mimic individual brain disorders or domains (Crawley, 2000; Sousa et al., 2006), neurophenotyping research may benefit from using alternative strategies to address the existing challenges. Here, we will argue that a wider use of "hybrid" models that comprehensively assess multiple behavioral domains may be instrumental in achieving these goals.

2. Methodological considerations

Although it is crucial that researchers avoid basing their interpretations of behavioral data on individual tests or domains (Crawley, 1999; Crawley, 2000), investigators interested in a particular trait sometimes perform a very restricted behavioral analysis, limited to the domain of interest (Tecott, 2003). The importance of an in-depth assessment of multiple domains for correct interpretation of neurobehavioral data has been recognized in the literature; see Tecott (2003) for review. One solution to optimize the throughputfulness of the experiments is to use behavioral models that allow the researchers to register as many parameters as possible. For example, the elevated plus maze test of anxiety targets several different domains (exploration, activity, risk assessment) and can be used for their simultaneous assessment in animals (Doremus et al., 2006; Walf and Frye, 2007).

Another solution, as already mentioned, is the use of batteries of specialized tests (Fig. 1A) that focus on different domains. At this stage, however, it is crucial to consider how behaviors can be affected by the previous testing experience of the animal, and what measures should be taken to ensure that the data are not compromised as a result. For example, timing is an important issue. Some studies indicate that mice respond differently when tested in a battery rather than in individual tests alone (McIlwain et al., 2001), showing that some behavioral tests are more susceptible to previous experience of the animal, while others are not. Other studies suggest that the inter-test time interval has little effect on overall performance (Paylor et al., 2006), which opens the opportunity for accelerated research techniques. Moreover, if one test does alter behavior in another, that fact does not disqualify the test from further use. In fact, the combination of the tests may provide opportunities for eliciting clinically relevant behaviors that could not be achieved with either test alone.

In addition to the test batteries' effect on animal behaviors, the nature of behavioral tests *per se* may sometimes preclude them from being able to form a battery. For example, the

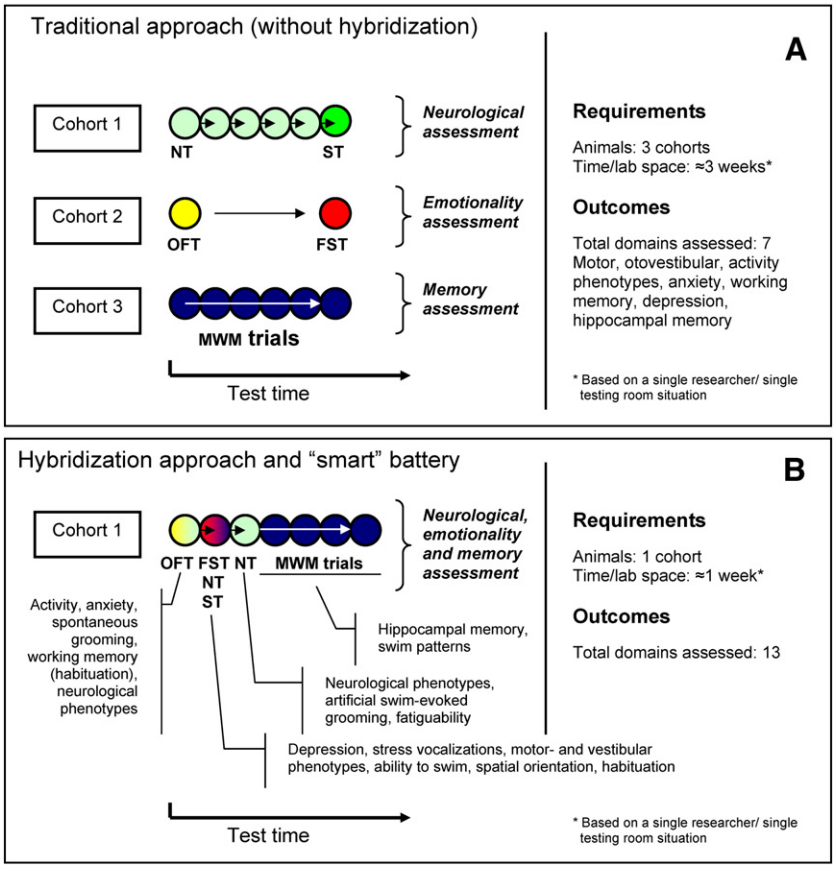

Fig. 1. Traditional neurophenotyping approaches (A) and the use of a combination of novelty-, activity- and swim-based tests to create a "smart" battery of "hybrid" tests (B) that helps maximize animal behavioral information. NT—different neurological tests; ST—swim test (ability to swim); OFT—open field test; FST—Porsolt's forced swim test, MWM—Morris water maze (see text for details).

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traditional use of various swim tests does not allow investigation of depression (assessed by immobility in the Porsolt's forced swim test (Cryan and Holmes, 2005)), neuromuscular abilities (assessed by the ability to swim in the water tank (Kalueff et al., 2004)) or hippocampal memory (assessed in Morris water maze; (Wolfer et al., 1997)) in the same cohort of animals due to their habituation to the swim situation. Thus, assessment of these domains individually will require at least three separate cohorts of animals and considerable testing time, as well as human and laboratory resources (Fig. 1A).

The hybridizing approach developed here offers a conceptually different perspective, based on specific "hybrid" protocols that either assess several different domains simultaneously, or logically combine several single-domain tests in a very special way, in order to maximize the number of phenotypes or domains they collectively measure (Fig. 1).

3. Hybrid models: examples and general overview

Several examples may illustrate this approach. A recently developed model shows how two different domains (i.e., anxiety and depression) may be targeted within a single experiment. In chicks, social separation evokes distress vocalizations as a behavioral response to re-establish social contacts (Sufka et al., 2006; Warnick et al., in press), and the pattern of this behavior changes as a function of length of separation stress. In the Sufka et al., (2006) study, chicks tested in the social condition vocalized little during the test session (Fig. 2). In contrast, isolated chicks displayed a significant increase in distress vocalizations which was maximal during the first 5 min block (anxiety-like state), significantly declined over the next 15–20 min (transitional phase) and stabilized at approximately 50% the initial rate for the remainder of the session (depression-like state). The benzodiazepine anxiolytic drug chlordiazepoxide attenuated distress vocalizations during the anxiety phase but did not affect these responses thereafter. In contrast, the tricyclic antidepressant imipramine decreased vocalizations during the anxiety-like phase and increased them during the depression-like phase (Fig. 2). Recent studies (Warnick et al., in press) have also shown that a wide range of

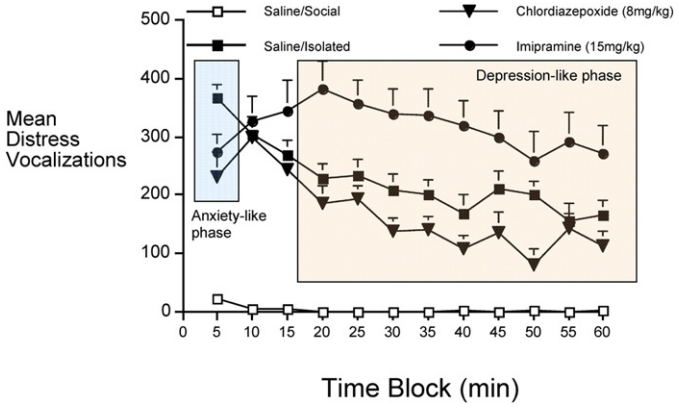

Fig. 2. Behavioral characterization and pharmacological validation of the chick anxiety-depression continuum model; adapted from Sufka et al. (2006).

anxiolytic and antidepressant compounds affect distress vocalizations in these ways in the two phases of the stress paradigm. Collectively, these findings imply that two different states – anxiety and depression – can be modeled in some species within a single time-efficient behavioral protocol. In addition, this model possesses other attributes that favor its adoption as an early preclinical dual anxiolytic/antidepressant screening assay, as it uses a lower purchase cost animal (compared to rodents), tests at a young age (lowering total per diem costs), employs a behavioral index that can be automatically recorded, and uses simple experimental designs (Sufka et al., 2006; Warnick et al., in press).

A different study elegantly modified the marble burying test in C57BL/6 mice by measuring locomotor activity using a videotracking system (Nicolas et al., 2006). Comparing marble burying scores with traveled distance measures, this study dissected "anxiety" and "activity" domains and established their differential sensitivity to various drugs. Minimizing animal number needs, this modified procedure appears to be a valuable screen for anxiolytic compounds (Nicolas et al., 2006), also suggesting that similar approaches may be used for other existing paradigms (e.g., nestlet shredding or social interaction; Table 1). Some other examples are summarized in Table 1, showing how behavioral models may easily be used more efficiently to simultaneously target multiple different domains.

Consider, for example, the situation described in Fig. 1. Indeed, a short pre-swim open field testing (Step 1) allows researchers to assess baseline anxiety and activity/motor phenotypes (Burne et al., 2006), novelty-evoked grooming behavior (Kalueff et al., 2007a), within-trial habituation (spatial working memory) and potential behavioral perseverations, such as meandering/turning or stereotypic circling (Kalueff et al., 2007c). Step 2 of this "smart" battery (Fig. 1B) includes an acquisition trial of the Morris water maze, a necessary step in this model, used as a Porsolt's forced swim test, as suggested recently (Schulz et al., 2007a,b). By examining depression-related immobility during this trial, this will enable a parallel assessment of depression-like behaviors without affecting the Morris water maze procedure. In addition to these domains, analyses of per-minute distribution of animal activity will assess their within-trial habituation (spatial working memory). Likewise, poor swimming during this trial will be indicative of motor/neuromuscular problems, whereas frequent circling and sinking, if present, may suggest otovestibular phenotype in these animals (Kalueff et al., 2008a, 2004). Aberrant turning/navigation and meandering in this situation may suggest altered spatial strategies. Finally, swim stress-evoked ultrasonic vocalizations (Fride et al., 2005) that may be measured in this test as stress-related indices.

In most cases, at this stage researchers will routinely remove animals from the water tank, dry them out and return to their home-cages. However, several additional domains may be assessed at Step 3, using the "smart" battery approach (Fig. 1B). For example, placing these mice for 5 min in the observation cylinder immediately after the swim test will enable the assessment of a different type of grooming behavior—the "artificial" swim-induced grooming (Burne et al., 2006). In case

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Table 1
Selected examples of "hybrid" (multi-domain) animal protocols for behavioral phenotyping research

Tests (models)	Domains that can be targeted within the same model	References
Stress hyperthermia	Baseline temperature assay, anxiety	(Olivier et al., 2003), also see: (Sufka and Hughes, 1991)
Open field	Activity, anxiety, habituation, spontaneous episodic memory, home-base formation, circadian rhythms, OCD-like perseverations	(Clark et al., 2006; Crawley, 2000; Kalueff et al., 2007c; Nemati and Whishaw, 2007)
Startle response	Hearing, anxiety, memory (habituation)	(Dulawa et al., 1997; Sousa et al., 2006; Tarantino et al., 2000)
Y- or T-maze	Spontaneous alternation, spatial memory, anxiety, OCD-like phenotypes	(Deacon and Rawlins, 2006; Tsaltas et al., 2005; Yadin et al., 1991)
Plus maze discriminative avoidance test	Memory, anxiety, motor activity	Kameda et al. (2007)
Social defeat paradigm	Anxiety, depression, aggression, social behavior	Avgustinovich et al. (2005)
Social interaction	Anxiety, activity, aggression, autism-like phenotypes	(Crawley, 2000; Holmes et al., 2002; Jaubert et al., 2007; Kalueff et al., 2007b)
Chick separation stress	Anxiety, depression, stress-evoked analgesia	(Feltenstein et al., 2002; Feltenstein and Sufka, 2005; Feltenstein et al., 2004; Sufka and Weed, 1994); Fig. 2
Suok test	Anxiety, balancing, motor activity, stress-evoked sensorimotor disintegration	(Kalueff et al., in press; Kalueff et al., 2005; Kalueff and Tuohimaa, 2005)
Grooming sequencing	Anxiety, OCD-like phenotypes, Tourette's syndrome-like phenotype	(Berridge et al., 2005; Kalueff and Tuohimaa, 2004)
Marble burying	Anxiety, motor activity, OCD-like phenotype	(Li et al., 2006; Londei et al., 1998; Nicolas et al., 2006)
Tail suspension test	Depression (immobility), vestibular abnormalities (spinning), specific neurological phenotypes (e.g., clasping). Can be combined with other tests (e.g., stress hyperthermia)	(Curtin et al., 2003; El Yacoubi and Vaugeois, 2007; Lalonde, 1987)
Compulsive drug intake	Reward, drug abuse phenotype, OCD-like phenotypes	Kenny (2007)
Stress neophagia	Feeding, anxiety, screening of drugs. Can be combined with the food finding test (measuring olfactory abilities of animals)	(Bodnoff et al., 1988; Dulawa and Hen, 2005)
Wheel running	Motor activity, circadian rhythms, social stress, anxiety	(Dishman et al., 1996; Hunter et al., 2000; Uchiumi et al., in press)
Food restriction-evoked hyperactivity	Motor activity, circadian rhythms, screening of drugs	(Altemus et al., 1996; Altemus et al., 1993; Yokoyama et al., 2007)
Water maze	Motor/neurological phenotypes, spatial memory, learned depression (immobility), vestibular phenotypes	(Schulz et al., 2007a,b), see Fig. 1B for details
Swim tests	Motor/neurological phenotypes, depression, vestibular phenotypes (sinking), spatial strategies, fatigueability	(Brooks et al., 2005; Burne et al., 2006; Kalueff et al., 2004), see Fig. 1B for details
Nestlet shredding test	Motor/neurological phenotypes, nest building, sensitivity to anxiolytics and antidepressants	Li et al. (2006)
Novel object recognition	Memory, anxiety (neophobia)	(Ennaceur et al., 1989; Ennaceur and Delacour, 1988)
Chronic stress paradigm	Anhedonic depression, anxiety, motor activity	(Strekalova et al., 2006, 2004, 2005)

Note that most of these procedures require only few additional endpoints or slight alterations of the traditional validated protocols. OCD—obsessive–compulsive disorder.

the animal's grooming phenotype was affected, this simple experiment may eventually lead to interesting findings about sequential organization of animal grooming by comparing activity and sequencing of pre-swim "spontaneous" grooming with the "artificial" swim-induced grooming. Furthermore, as has recently been demonstrated (Burne et al., 2006), by comparing pre- and post-swim behavioral activity levels, some conclusions may be made about animal fatigueability—another important domain that merits scrutiny in neurophenotyping research (Fig. 1B). Finally, Step 4 of this battery includes subsequent trials of the Morris water maze that, according to the traditional protocol, assess the animals' spatial memory (Crawley, 1999; Paylor et al., 1999).

4. Problems, limitations and solutions

Importantly, the proposed phenotyping strategy has some limitations, and the researchers must be aware of them in order to further improve their research. For example, the issue of generalizability and interpretation of results can become very complex when using hybrid modeling techniques, as the domains that are being screened may not be discrete at the neurobehavioral levels, and an animal's reaction to the given "hybrid" test conditions could be different than in any of the single-domain paradigms (e.g., (McIlwain et al., 2001)). For example, in the elevated plus maze, the anxiety induced by novelty and the open areas of the arms may inhibit the learning and memory processes of the animal, thereby making it difficult to derive meaningful data in that behavioral realm. However, these possible confounding factors can be attenuated and pose less of a concern through the use of appropriate strains and slight experimental modifications.

Procedural differences in experimental manipulations and other "external" factors may also be an important problem. For example, the initial session of the Morris water maze may be appropriate as a preliminary screen for depression-related behavior (Schulz et al., 2007a), extreme differences in the testing arenas and protocols used in these tests introduce

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variables that could significantly impact comparisons of "hybrid" behavioral endpoints with standard single-domain or standard test battery protocols. In mice, the typical trial time for the water maze is between 1 and 2 min, while the forced swim test duration is usually around 6 min to ensure that adequate levels of immobility can be observed (with scoring of behavior often limited to the final 4 min, when behavioral immobility begins to emerge). In this comparison, mice in the "hybrid" protocol may display abnormally low behavioral immobility due to the brief session duration. Nevertheless, there are also ways to reconcile these differences without compromising the legitimacy of the model. For example, to protect against any potential experimental artifacts, an investigator may choose a trial that exemplifies an appropriate compromise between the two. For instance, a mouse could be run for 5 min in each swimming paradigm, thereby allowing enough time for learning to occur and the behavioral immobility to be observed, yet not so much time that the mouse becomes unduly fatigued.

Likewise, the temperature can vary from paradigm to paradigm, and may also impact behavioral performance in the swim tests (Bachli et al., 2008; Iivonen et al., 2003). Therefore, when performing a hybrid model, it is important to select the temperature that best fits the model as a whole, to minimize possible confounds. Similar solutions, aiming at standardizing testing conditions across the battery, may be an effective way to improve the validity and reliability of neurophenotyping findings.

In a similar vein, important behavioral information can be obtained by correlating specific endpoints in hybrid models (e.g., the swim speed in the water maze with activity levels in the open field test). Indeed, some rodent mutants show significantly different responses (than their wild type littermates) in the swim test, while activity levels in the open field test may remain unaltered (Redrobe et al., 2004). If an investigator relies on the open field test alone, he may have an inaccurate or incomplete impression of the rodent immobility during swimming. From this point of view, the hybridization strategy provides an efficient and simple solution to this phenotyping problem.

5. Conclusions

Certainly, we do not propose to fully replace the existing single-domain experimental models with new "hybrid" paradigms. However, the examples presented in Table 1 and Figs. 1 and 2 clearly demonstrate that behavioral phenotyping may benefit markedly from intensifying research by including a larger proportion of multi-domain models in the experimental repertoire.

Is this approach a difficult undertaking? Apparently not, as in many cases this does not require major procedural modifications, and can be easily implemented by simply adding several extra endpoints that target a different domain (Table 1). For example, examining grooming sequencing in standard open field anxiety/activity test can detect OCD-like phenotypes (Kalueff et al., 2007a), whereas assessing per-minute distribution of explorations turns this protocol into a simple spatial memory/habituation task (Leussis and Bolivar, 2006, Table 1). Although this approach requires some logistical efforts from the researchers, it may also lead to interesting new findings (aberrant grooming, affected habituation) that would otherwise remain undiscovered.

Other ways to obtain multi-domain models include the use of traditional single-domain models in novel contexts (e.g., Morris water maze as Porsolt test; Fig. 1B) or combining several models to target additional domains and their interplay (e.g., (Nicolas et `al., 2006; Slotkin et al., 1999)). Finally, the development of principally new multi-domain models that target clinically relevant neurobehavioral phenomena (Einat, 2006; Kalueff and Tuohimaa, 2005; Sufka et al., 2006) may be necessary to warrant further progress in this field.

In general, assessing more domains per experiment, "hybrid" models would require fewer stress exposures (than a combination of single-domain models), and therefore will adhere to the 3Rs [replace, reduce, refine] principle (Wurbel, 2007) by reducing animal numbers and suffering. Some "hybrid" models mentioned here, such as the chick social separation model, further implement the 3Rs principle by reducing the number of purpose-bred research animals (e.g., male chicks are a by-product of the commercial egg-laying industry) and replacing the standard rodent-based models with a phylogenetically lower and, perhaps, less sentient species (Sufka et al., 2006; Warnick et al., 2006).

Secondly, by targeting more domains and utilizing more behavioral endpoints per experiment (Table 1, Figs. 1 and 2), these models help save time and laboratory resources, thus emerging as efficient, high-throughput and less expensive screens. Thirdly, the use of batteries with fewer (but more extensive) tests reduces the impact of prior stress on subsequent behaviors (that may be potentially conditionable), therefore ensuring less confounded and more valid behavioral data. Fourthly, the simultaneous focus of "hybrid" models on distinct domains (such as anxiety and depression) enables mimicking of clinically relevant phenomena (comorbidity) that are difficult or impossible to target in single-domain models. Fifthly, by examining a wider spectrum of behavioral phenomena, hybrid models are more likely to "net" novel/complex phenotypes, especially when screening drugs or mutant animals with unknown or unclear profiles. Lastly, the use of such models enables a better focus on the newly appreciated "continuum" nature of brain pathogenesis (Akiskal and Benazzi, 2005; Lara and Akiskal, 2006; Warnick et al., in press), thus fostering further innovative translational research and integrative modeling of brain disorders. Collectively, this supports the developing utility of the "hybridization" strategy, which may become a solution for some of today's challenges in neurophenotyping research.

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