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High-throughput analysis of behavior for drug discovery

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ABSTRACT

Drug testing with traditional behavioral assays constitutes a major bottleneck in the development of novel therapies. PsychoGenics developed three comprehensive high-throughput systems, SmartCube®, NeuroCube® and PhenoCube® systems, to increase the efficiency of the drug screening and phenotyping in rodents. These three systems capture different domains of behavior, namely, cognitive, motor, circadian, social, anxiety-like, gait and others, using custom-built computer vision software and machine learning algorithms for analysis. This review exemplifies the use of the three systems and explains how they can advance drug screening with their applications to phenotyping of disease models, drug screening, selection of lead candidates, behavior-driven lead optimization, and drug repurposing.

1. Introduction

Neuropsychiatric, developmental and neurodegenerative disorders are complex and involve multiple neuronal circuits. Target-based approaches have, for the most part, failed to deliver meaningful treatments, whereas phenotypic screening has proved more successful. In the period between 1999 and 2008, 75 first-in-class drugs with novel mechanism of action were approved. Of the first-in-class drugs, 28 were discovered using phenotypic screening vs. 17 using target-based approaches. Specifically in CNS, 7 of the 8 first-in-class drugs approved were discovered using phenotypic screening (Swinney and Anthony, 2011).

It is not surprising, therefore that many of the most efficacious drugs, especially in psychiatry, have multiple targets and were discovered by serendipity (observing how an animal's behavior was altered in response to the drug). Since the goal of any neuropsychiatric drug is to impact behavior, PsychoGenics has industrialized "serendipity" with its behavior-based technologies.

PsychoGenics' proprietary behavior-based technologies, also known as the *SmartCube®*, *NeuroCube®* and *PhenoCube®* systems, combine behavioral neurobiology insight integrated with advances in robotics and computer vision (video capture and analysis) and the power of bioinformatics to process and analyze massive temporal and vectorial datasets using probabilistic causal inference algorithms (Fig. 1). The technologies offer numerous distinct advantages over current behavioral assessment including the following:

Highthroughput—can screen tens of thousands of compounds for CNS activity and identify those with a behavioral profile that reverses a disease model phenotype or is reminiscent of drugs that treat a specific neuropsychiatric disorder;
High content—thousands of features are collected and proprietary bioinformatics algorithms are employed to detect subtle phenotypic differences associated with a disease model or drug effect.
Unbiased—Computer vision algorithms and bioinformatics eliminate human intervention and subjectivity.

PsychoGenics uses its platforms at all stages of drug discovery as described below, to identify novel treatments addressing major unmet neuropsychiatric disorders that are unlikely to be found by other means. Platform applications include

- Screening representative compounds from diverse CNS libraries. This approach is agnostic to compound mechanism of action;
- Re-purposing compounds that are discontinued (for reasons other than safety) or currently being developed for other non-CNS indications;

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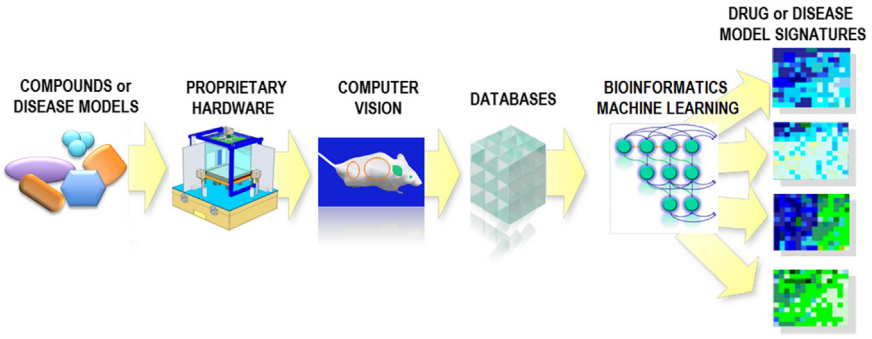

Fig. 1. SmartCube® combines behavioral neurobiology insight integrated with advances in robotics and computer vision (video capture and analysis) and the power of bioinformatics to process and analyze massive temporal and vectorial datasets using probability causal inference algorithms. SmartCube® is a platform that provides a sequence of challenges to a mouse, extracts more than 2000 features during a session and using proprietary bioinformatics detects the potential of compounds to treat psychiatric disorders in an unbiased way by comparing their complex behavioral profiles with those from a proprietary reference database.

- Screening target-focused compounds to determine the therapeutic utility of a target or identifying a preferred chemotype;
- Assessing compound combinations (i.e. determining the efficacy of a combination of novel compounds or a novel compound combined with an existing marketed drug);
- Lead optimization.

Using this approach, PsychoGenics has identified several drug candidates at various stages of clinical and preclinical development on its own and in partnership with other companies.

2. The SmartCube® system

The SmartCube® system is a high-throughput automated behavioral platform that presents a sequence of challenges to a mouse through its customized hardware, extracts more than 2000 features per session, and, using proprietary bioinformatics, and detects the potential therapeutic efficacy of compounds.

SmartCube® employs computer vision and mechanical actuators to detect spontaneous and evoked behavior eliciting responses through anxiogenic and startling stimuli. Behavioral readouts include locomotion, trajectory complexity, body posture and shape, simple behaviors and behavioral sequences (Brunner et al., 2002; Houghten et al., 2008; Roberds et al., 2011). Supervised machine learning algorithms are used to analyze the collected features. Although approximately ½ million datapoints are collected per mouse per session, behavioral definitions, machine learning techniques and smart voting under uncertainty, are used to reduce this dataset to ~2000 target features.

PsychoGenics' proprietary supervised machine learning methodology, derived from minimization of Bayesian misclassification probability, similar in spirit to Support Vector Machines, is used to train a classification algorithm that reliably maps behavioral features for each drug to its corresponding biological response "label" (e.g. CNS Indication or Mechanism of Action). The original feature space undergoes non-linear transformation using a proprietary semi-blind source separation variant of Independent Component Analysis to minimize "overcounting", during calculation of the contribution of overrepresented original features, and reduce the effective (new) feature dimensionality. The output of the resulting classification algorithm is a probability distribution over the chosen set of labels which, in addition to a specific biological response, predicts quantities such as "unknown activity" (difference from vehicle not attributable to any specific feature patterns in the training set) as well as "total activity" of the drug (Fig. 2).

Two major types of analyses are routinely conducted: class and subclass. For class and subclass analyses, a reference data set has been built from hundreds of drug doses grouped in multiple drug classes plus a vehicle class. Dose responses for the reference drugs were constructed using multiple doses targeting both efficacious doses as well as doses that exhibit side effect profiles in mice. The class analysis uses labels and corresponding drugs that are currently in the market or have been clinically validated for a specific therapeutic indication. The Subclass analysis uses labels and a larger set of compounds selected from both marketed drugs and compounds validated for specific therapeutic uses. The reference databases are continually expanding with the addition of novel therapeutics and new proprietary databases are currently in development.

Novel compounds can be tested in SmartCube® system and the results can then be compared to the signatures of reference compounds in PsychoGenics' database. Multiple analyses of the data are performed to quantitatively produce independent predictions of drug class, and drug subclass. The system, therefore, can, in an unbiased way classify compounds according to the therapeutic potential by comparing their complex behavioral profiles with those from a proprietary reference database.

The results for the class and subclass analyses are presented as standardized bar charts with percentages that sum to 100 for each dose. The results of the classification at the drug level are presented as individual similarities. An example of the output of a typical classification is shown in Fig. 2A.

Fig. 2B shows a different use of the system, as a full profile appears when a large dose response is run, in this case for diazepam. The drug goes from inactive at .25 mg/kg to anxiolytic between 1.0 to 2.0 and sedative at higher doses. In this way, therefore, a therapeutic window and complete profile can be established for any drug. An interesting characteristic of the total pharmacological activity represented by the height of the colored bar is that it captures all beneficial, neutral, detrimental and unknown effects of the drug, so it continues to grow as the dose increases, but the color profile changes indicating the changing nature of the pharmacological action.

Fig. 2C depicts one of the first projects that benefited from the SmartCube® system. Different psychostimulant drugs are shown and compared against Eltoprazine. Despite Eltoprazine being from a very different class (a partial 5HT1A/1B agonist) it showed similarity to drugs used in Attention Deficit Hyperactivity Disorder (ADHD). Using these results and other preclinical experiments that confirmed activity of this compound in attenuating hyperactivity and impulsivity in various animal models, PsychoGenics conducted a proof-of-concept study in adults with ADHD. The study showed both doses tested (5 mg bid and 10 mg, bid) significantly improved ADHD symptoms using the ADHD rating scale (The Foundation for Medical Practice Education, www.fmpe.org, 2008) as compared to placebo ($p < .003$ and .037, respectively).