Research Article

Neurology Research & Reviews

Establishing a Quantitative Phenotype for Craving: Introducing a Novel Parametric Assay into Currently Qualitative Preclinical Frameworks

Francesco Crespi*

Indipendent researcher, Verona, Italy

***Correspondence Author:**
Francesco Crespi, Indipendent researcher, Verona, Italy.
E-mail: fm.crespi@libero.it

Citation: Francesco Crespi. Establishing a Quantitative Phenotype for Craving: Introducing a Novel Parametric Assay into Currently Qualitative Preclinical Frameworks. *Neurol Res Rev.* 2026; 1(1), 01-04.

Received Date: 01 July 2026 **Accepted Date:** 06 July 2026 **Published Date:** 10 July 2026

Summary

A novel, non-invasive, two-phase behavioral assay is introduced to quantify drug craving in rodents, bypassing surgical catheterization. Phenotypic screening of a baseline population (N = 53) via two-bottle choice successfully stratifies rats into Drug-Preferring and Water-Preferring subgroups using an optimized 0.5 mg/mL cocaine concentration. A representative sub-sample is then tested in an apparatus where an adjustable, spring-loaded door acts as a physical analogue to a progressive-ratio schedule. Under increased mechanical resistance (150g to 300g, corresponding to 1.47 to 2.94 Newton, and 0.147 to 0.294 Joule over a 10 cm door displacement), drug-preferring subjects exhibit persistent motivation to re-enter the drug chamber, whereas controls cease crossing. Notably, water-preferring rats actively reject the drug after a brief sip, returning immediately to the neutral water chamber. This refined protocol transforms qualitative profiles into a precise, scalar, and quantitative metric of motivational drive determined by the animal's internal craving state, maintaining veterinary refinement to attain reduction-refinement-respect, the three Rs towards ANIMAL WELFARE in preclinical research.

Keywords: Drug craving quantification, Preclinical behavioral models, Non-invasive operant paradigms, Spring-loaded door assay, Two-bottle choice screening, Progressive-ratio analogue, Animal welfare and refinement.

Introduction and Classical Behavioral Paradigms

Understanding the complex neurobiological architecture of Substance Use Disorders (SUDs) relies heavily on the translation of clinical diagnostic criteria into reliable preclinical rodent models (Ahmed, 2009). Over the past several decades, behavioral neuroscientists have developed distinct experimental paradigms designed to isolate and quantify specific phases of the addiction cycle, namely reward valence, operant reinforcement strength, and the propensity for relapse (Sanchis-Segura & Spanagel, 2006).

Among the non-contingent procedures, the Conditioned Place Preference (CPP) paradigm remains a cornerstone for evaluating the passive, Pavlovian associative learning mechanisms linked to drug reward (Tzschentke, 2007). In a typical CPP setup, animals learn to pair the distinct environmental context of a specific chamber with the interoceptive effects of a drug, while an alternative chamber is paired with a saline vehicle. The primary metric of interest is the significant increase in time spent in the drug-paired environment during a subsequent, drug-free post-test. Because the animal is tested in an acute drug-free state, CPP is highly regarded for its utility in screening the implicit rewarding or aversive properties of novel compounds without the confounding variable of active drug consumption (Sanchis-Segura & Spanagel, 2006; Tzschentke, 2007). However, a notable limitation of this paradigm is its inability to model volitional drug-seeking behavior or active operant reinforcement.

To address the active components of drug-taking, the Intravenous Self-Administration (IVSA) paradigm serves as the gold standard for modeling volitional intake and operant reinforcement pathology (Panlilio & Goldberg, 2007). Utilizing jugular-catheterized subjects, IVSA allows animals to actively execute a response—such as a lever-press or a nose-poke—to trigger automated intravenous drug infusions. When evaluated under a Fixed-Ratio (FR)

schedule of reinforcement, where a stable number of responses is required for each dose, researchers can assess baseline consumption and primary reinforcement dynamics. Conversely, to measure the exact economic demand and incentive motivation an animal possesses for a substance, a Progressive-Ratio (PR) schedule is employed (Panlilio & Goldberg, 2007). Under a PR schedule, the response requirement escalates exponentially for each successive dose. The final completed ratio before the animal ceases to respond, known as the breakpoint, provides a direct quantitative index of the subject's motivation to obtain the drug.

Finally, to bridge the gap between active intake and clinical relapse, the field relies heavily on Extinction and Reinstatement models (Bossert et al., 2013). Following a period of stable drug intake, the active operant responses no longer yield drug delivery, causing the behavior to undergo extinction and gradually decay. Once a low baseline of responding is achieved, drug-seeking behavior can be forcefully re-initiated using specific clinical relapse triggers—such as drug primes, acute stressors, or environmental sensory stimuli previously paired with drug delivery—all without re-introducing active drug infusions (Bossert et al., 2013).

While these classical reinstatement models provide critical insights into relapse liability, they introduce a significant translational gap. Primarily, they typically assess drug-seeking only after artificial extinction training—a process that rarely mirrors human addiction, where environmental cues remain highly salient during abstinence. Furthermore, these paradigms often struggle to isolate pure, ongoing craving states in multi-choice environments where adaptive, natural rewards are simultaneously available (Banks & Negus, 2017), and the field has long lacked an objective, parametric methodology to numerically evaluate the psychological intensity of this drive. This methodological gap necessitates a shift toward protocols that can evaluate the actual burden of craving without extinguishing cue-drug associations. To overcome these limitations, we introduce a novel behavioral assay specifically designed for quantifying craving. By measuring the exact motivational cost and punishment resistance an organism is willing to endure, this protocol transforms an elusive psychological state into a highly precise, measurable metric.

Introduction of a Novel Non-Invasive Paradigm for Assessing Craving

While traditional operant models like IVSA provide robust quantification of drug motivation, they heavily rely on invasive, costly, and resource-intensive surgical catheterization protocols that inherently compromise animal welfare and introduce physiological confounding variables. To bypass these limitations, the present work introduces a novel, entirely non-invasive behavioral framework designed to screen individual vulnerability and isolate drug-craving states through a multi-phase, non-surgical paradigm. By eliminating the need for chronic surgical maintenance and reducing catheter-related health complications, this protocol represents a significant veterinary refinement in line with the ethical mandates of preclinical research i.e. to attain reduction-refinement-respect, the three Rs towards ANIMAL WELFARE in preclinical research (Crespi, et al. 2019).

BEHAVIORAL FRAMEWORK

[Phase 1: Two-Bottle Choice Screening]
(Water vs. Oral Psychotropic Drug)
▼
[Phenotypic Stratification of Sample]
(Drug-Preferring vs. Water-Preferring)
▼
[Phase 2: Resistance-Door Testing]
(Quantification of Craving via Spring Tension)

The paradigm is divided into two operational stages:

1. Phenotypic Screening via Two-Bottle Choice (TBC): In the baseline phase, animals are exposed to a free-choice drinking paradigm in their home cages. One bottle contains standard vehicle (water), while the parallel bottle contains a diluted solution of the target psychotropic drug. By measuring the volumetric intake over a designated period, the baseline population **(N = 53)** is successfully stratified into distinct phenotypic subgroups: Drug-Preferring (DP) and Water-Preferring (WP) animals (for more methodological details see Crespi, 1998). Briefly, this approach distinguished between adult male rats (average weight 250g) that consumed water exclusively (approximately **34 mL**/session, **n = 32**) and those that consumed a significant amount of cocaine alongside water (**12 mL** of cocaine solution + **15 mL** of water, **n = 21**), with the total fluid intake remaining statistically similar between both groups.

The cocaine concentration was previously optimized by challenging the rodents with increasing doses of the substance, establishing an overall population preference for 0.5 mg/mL. This initial step effectively maps the putatively innate inter-individual sensitivity to the target substance without the need for complex operant pre-training (Crabbe et al., 2010; Sanchis-Segura & Spanagel, 2006).

2. The Spring-Loaded Door Craving Assay: Following the identification of the drug-preferring phenotype, a representative sub-sample of 5 WP and 5 DP subjects is introduced into a specialized testing apparatus divided into two distinct zones (FIGURE 1).

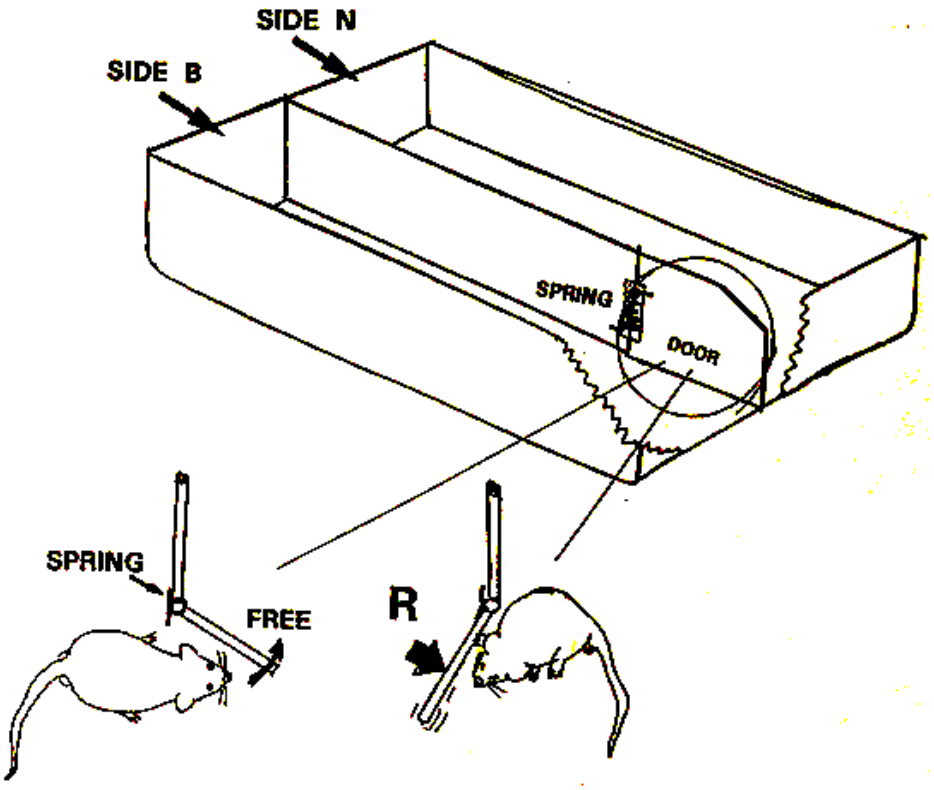

Figure 1: Experimental testing environment showing the neutral **(Side N)** and reward **(Side B)** chambers separated by the spring calibrated resistance door **(R)**.

Behaviour

For the behavioral test, lasting 15 min for each animal, a testing apparatus with two chambers separated by a door was used (see figure 1). The door opens freely in one direction, while an interchangeable spring of calibrated resistance forces the rodent to push to enter the second room, in which there is a bottle of the target psychotropic drug, i.e. cocaine, or water when testing DP rats (n=5) or WP rats (n=5), respectively. The first room is empty when the animal is inserted at the beginning of the test, so that it acts as a neutral side (SIDE N). When the animal enters the second room (SIDE B) for the first time, a bottle of water is placed in the first room. The time spent in the neutral side, the time spent in both rooms, the number of crossings through the door, and the consumption of liquid from the two bottles are the parameters considered.

These behavioral observations indicated that: DP rats crossed the door more rapidly for the first time than the WP rats. They drank mainly from the cocaine solution, while the WP rats consumed a similar amount of liquid from both water bottles and spent a similar amount of time in both rooms. In contrast, the DP rats remained in the cocaine side for approximately two-thirds of the session. Notably, during initial testing, when WP rats previously selected via the two-bottle choice encountered cocaine in Side B, they immediately returned to Side N to drink water after only a brief sip of the drug solution, confirming an active rejection of the substance.

In addition, the spring could be rapidly and easily replaced to increase the resistance from 150g to 300g. This shift corresponded to an increase in exerted force from 1.47 Newton to 2.94 Newton, and a mechanical workload increase from 0.147 Joule to 0.294 Joule over a full 10 cm opening displacement. Following the first return of the rats to the first room, only the DP rats exhibited the persistent motivation to overcome the higher force and re-enter the drug chamber, whereas WP rats ceased crossing (see Table 1)."

Table 1

Behavioral Parameter	Drug-Preferring (DP) Rats (n=5)	Water-Preferring (WP) Rats (n=5)
Initial Crossover Latency	90 ±12 sec.	192 ± 15 sec.
Spatial Preference (% of session time)	≈66.7% in Cocaine Side B	≈ 50.0% Equally distributed
Fluid Consumption in Side N	0.4 ± 0.12 ml Water	4.1 ± 0.9 ml Water
Fluid Consumption in Side B	8.9 ± 1.0 ml Cocaine	4.8 ± 0.6 ml Water
Resistance Breakpoint 150g -> 300g / 1.47Newton -> 2.94Newton	Persistent Crossed at higher force	Ceased No crossings observed

Note. DP: Drug-Preferring; WP: Water-Preferring. Side B contained cocaine for DP rats and water for WP rats. Evaluating WP subjects under identical resistance levels provides the essential baseline control for non-specific physical drive.

In conclusion, the zones N (NEUTRAL) and B (REWARD SIDE) are separated by a unidirectional access door that is mechanically restricted from one side by an adjustable spring-loaded tension system. To gain access to the zone containing the drug reward, the animal must physically push against the door, overcoming the mechanical resistance exerted by the spring. By systematically altering the spring tension, this setup functions as a mechanical and physical analogue to an operant progressive-ratio schedule, mirroring established preclinical paradigms that utilize physical barriers or weighted doors to assess incentive demand (Allain et al., 2017; Thomsen et al., 2016).

Importantly, because this mechanical resistance can be precisely calibrated, the apparatus allows for the direct quantification of craving intensity by measuring the exact physical force, expressed in Newtons, and the workload, expressed in Joules, that the animal exerts. This physical effort is causally determined by the subject's internal craving state and incentive motivation. Conversely, evaluating the water-preferring subgroup under identical conditions provides an essential baseline control to rule out generalized locomotor exploration and non-specific physical drive (Banks & Negus, 2017).

Future experiments will utilize a progressive sequence of calibrated springs—specifically 150g, 200g, 250g, and 300g—to map a fine-grained, scalar, and strictly quantitative phenotype of drug craving.

This paradigm offers a high-throughput, and ethologically sound alternative to classical reinforcement models, successfully isolating motivation and craving while upholding the highest standards of veterinary refinement to attain reduction-refinement-respect, the three Rs towards ANIMAL WELFARE in preclinical neuropharmacology.

Acknowledgments: The author would like to thank Francesco Congestri' for technical support and to Professor Paul Nuthall (University of Nottingham) for fruitful discussions.

References

1. Ahmed, S. H. (2009). Validation of animal models of drug addiction: a critique. Neuropharmacology, 56, 122–131.]
2. Allain, F., Minogianis, E. A., Roberts, D. C., & Samaha, A. N. (2017). Individual differences in the motivation to seek cocaine: a barrier-choice paradigm. Psychopharmacology, 234(11), 1695–1707.
3. Banks, M. L., & Negus, S. S. (2017). Insights from Preclinical Choice Models on Treating Drug Addiction. Trends in Pharmacological Sciences, 38(2), 181–194
4. Bossert, J. M., Marchant, N. J., Zanagnolo, D., & Shaham, Y. (2013). The reinstatement model of drug relapse: recent neurobiological findings and future directions. Neuropharmacology, 67, 24–34.
5. Crabbe, J. C., Harris, R. A., & Koob, G. F. (2010). The Two-Bottle Choice Test for Alcohol Consumption: Procedures and Genetic Control. Frontiers in Behavioral Neuroscience, 4, 15.
6. Crespi, F. (1998). The role of cholecystokinin (CCK), CCK-A or CCK-B receptor antagonists in the spontaneous preference for drugs of abuse (alcohol or cocaine) in naive rats. Methods and Findings in Experimental and Clinical Pharmacology, 20(8), 769–775.
7. Crespi, F., Formenti, F., & Congestri, F. (2019). Near infrared spectroscopy (NIRS) to attain reduction-refinement-respect, the three Rs towards ANIMAL WELFARE in preclinical research. Neurol Neurol Sci Open Access. 2019; 2(1): 1008
8. Panlilio, L. V., & Goldberg, S. S. (2007). Self-administration of drugs in animals and humans as a model and an assay of abuse liability. Addiction, 102(12), 1860–1870.
9. Sanchis-Segura, C., & Spanagel, R. (2006). Behavioral assessment of drug reinforcement and the effects of drugs on reward mechanisms. Neuroscience & Biobehavioral Reviews, 30(4), 147–178.
10. Thomsen, M., de肩, O., & Caine, S. B. (2016). Quantifying motivation in mice using a progressive ratio weighted-door apparatus. Journal of Neuroscience Methods, 273, 116–123.
11. Tzschentke, T. M. (2007). Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. Addiction Biology, 12(3-4), 227–462.