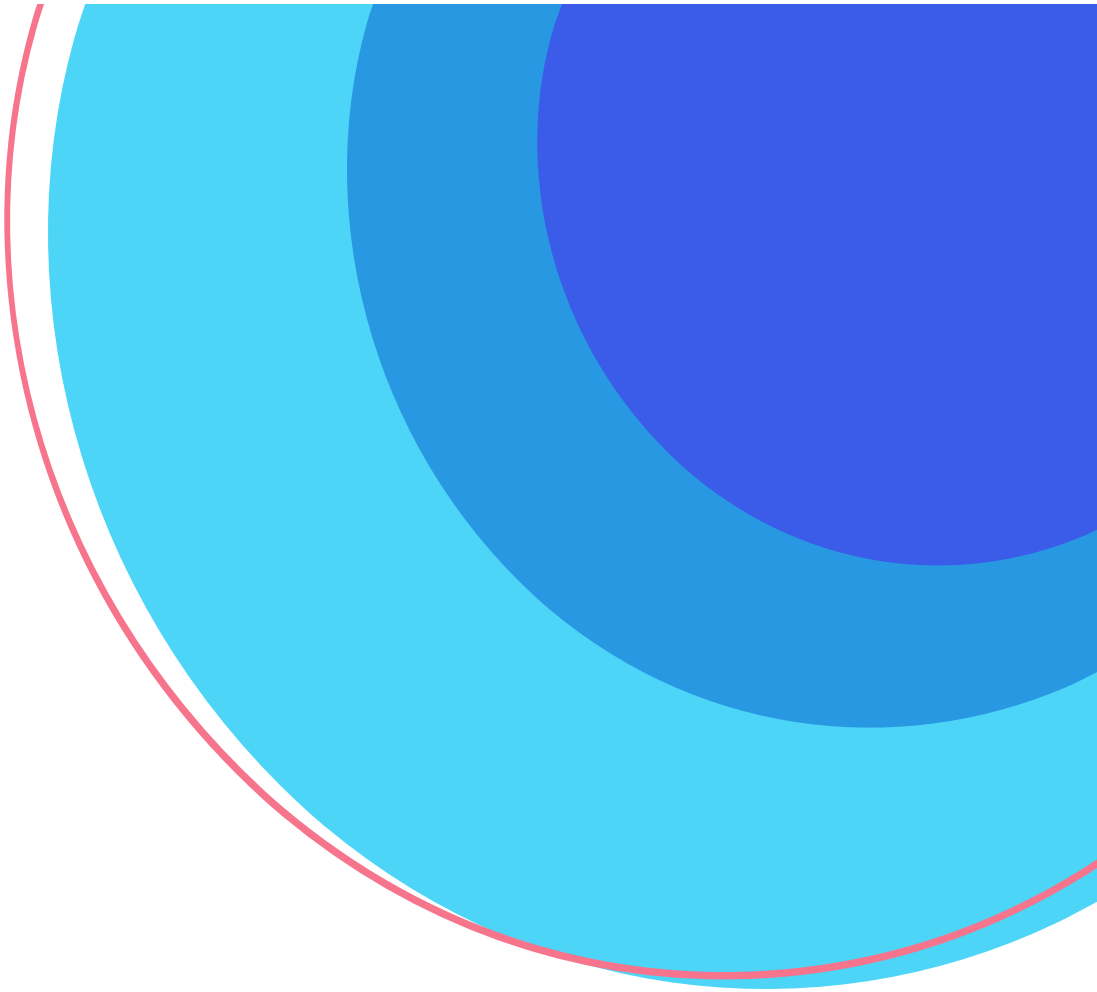
# Defining controls, variables and conditions for preclinical evaluations of a novel drug entity

Sharon Rowton, PhD



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# Intravenous self-administration (IVSA)

# General considerations (IVSA)

| ANIMALS | FORMULATIONS | DOSE LEVELS |
|---|---|---|
| Body weights | Soluble (fixed volume of 0.2 mL) | Consider in conjunction with PK and behavioural data |
| Minimise injury risk | pH – ideally between 4 and 9 | Fractions of the highest therapeutic clinical $C_{max}$ |
| Prompt treatment of surgery site sores | Stable at room temperature | Not anticipated to result in overdose if administered in quick succession |
| Handle animals with care | Compatibility with dosing equipment; Formulations may be in use for several hours | Do not impair lever pressing ability |
| Flush lines regularly to maintain patency | Vehicles should not affect lever pressing ability | Do not produce lasting effects that may reduce the frequency of administration |
| Employ aseptic procedures where possible to avoid the introduction of infection | Some vehicles can be ‘sticky’ | |
| | Aseptic methods | |

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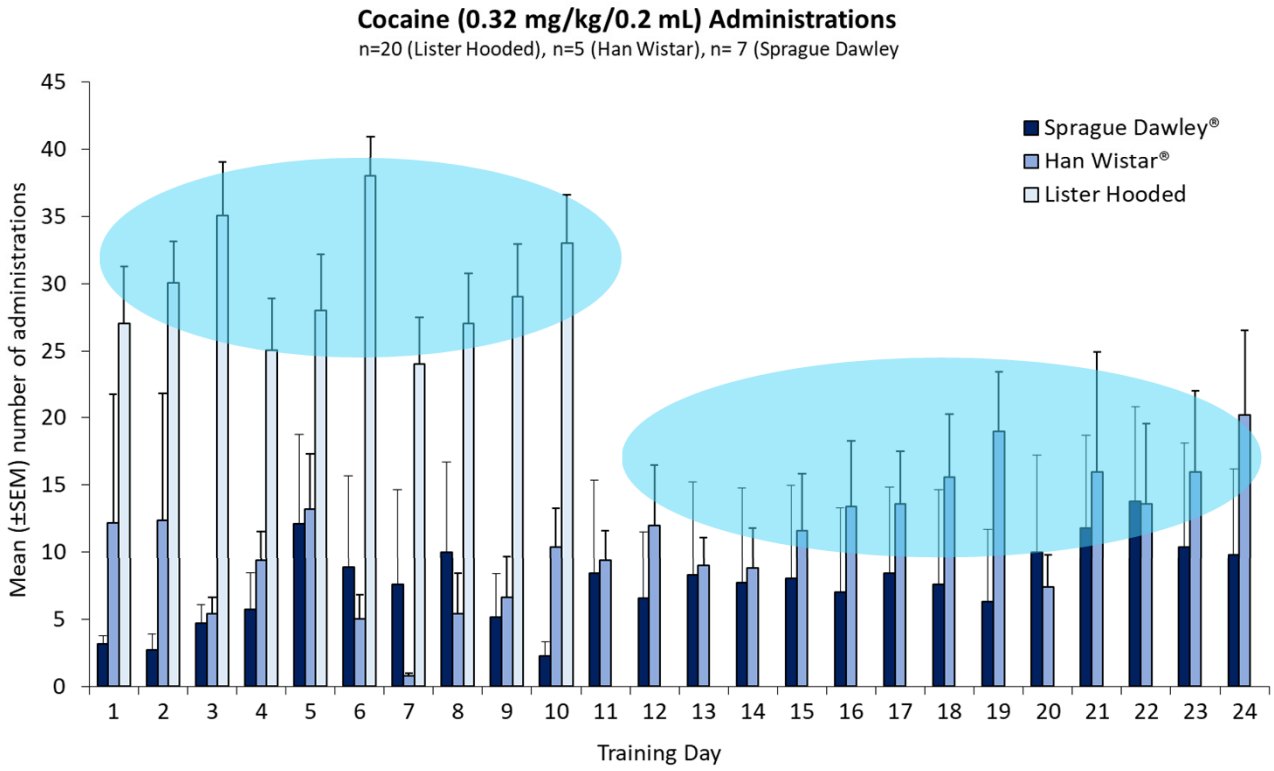
# Strain of rat (IVSA)

The strain of rat used is typically dictated by the strain used within the validation studies conducted at a particular laboratory.

Some strains may train more readily than others and this may account for the slight differences in designs between laboratories (e.g., FR, maximum administrations).




All Lister Hooded rats had progressed to an FR10 schedule of reinforcement by Day 7

By Day 24, only one Sprague Dawley rat was responding under an FR10

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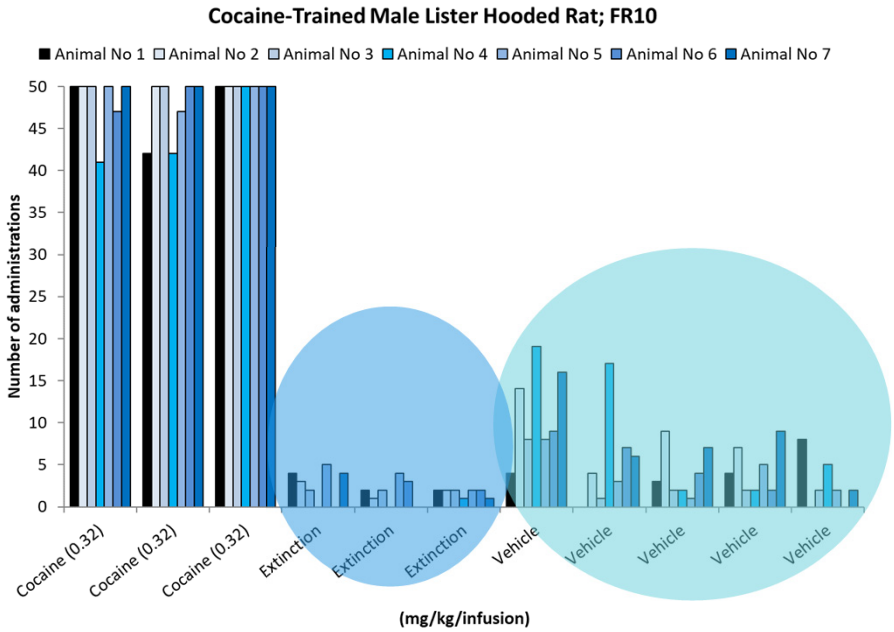
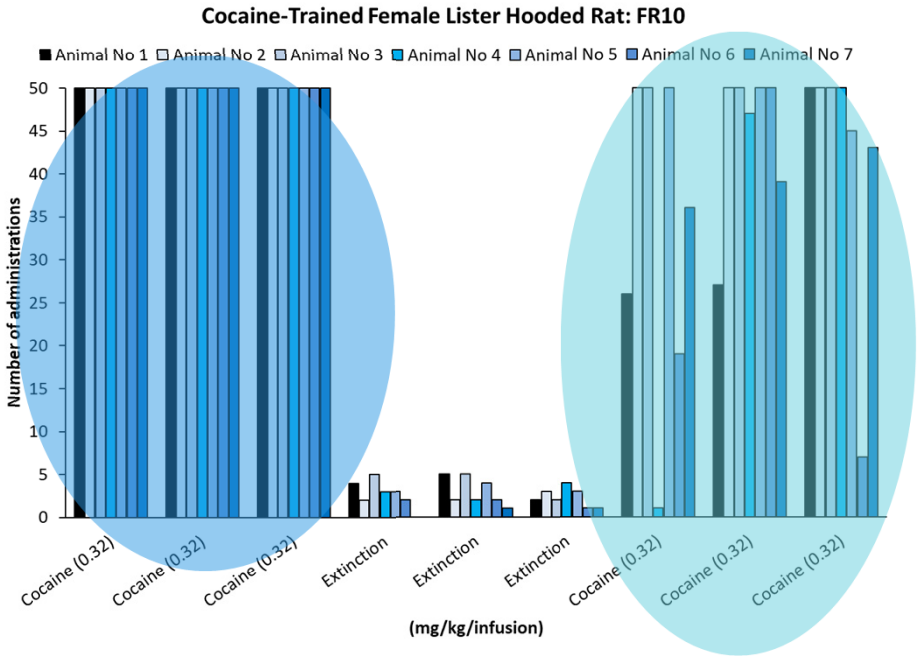
# Controls (IVSA)

**Vehicle control**
Under test conditions, vehicle responding is rarely comparable to the ‘forced’ values of extinction.




**Positive control**
Final training sessions are ‘forced’ values.
Under test conditions, i.e., following extinction, responding may be different.

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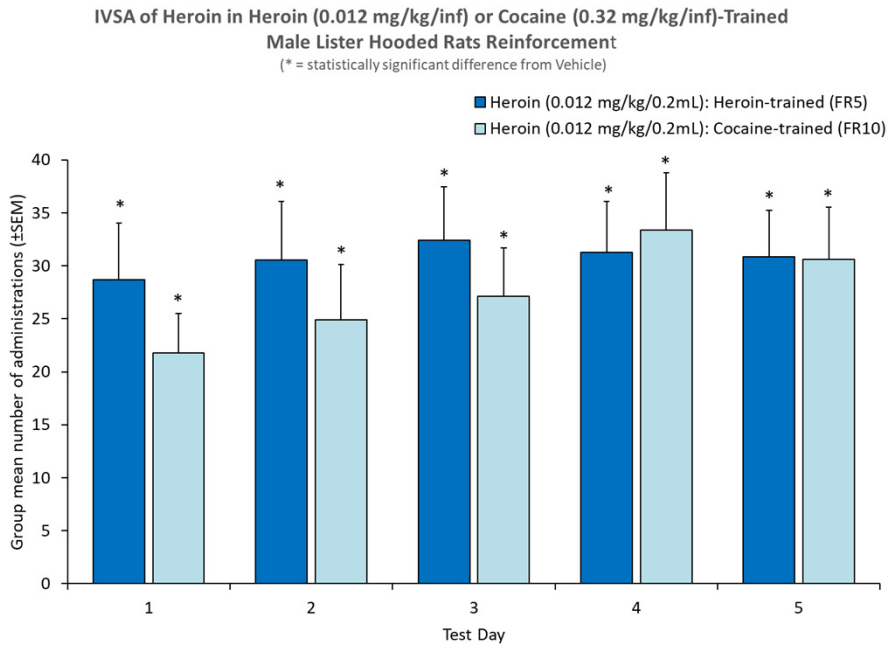
# Training drug (IVSA)

The primary purpose of the training drug is to see if animals can acquire lever pressing behavior in response to a *reward.*

This learning is then adopted to the self-administration of the test article if it is perceived as being rewarding.

Although acquisition of heroin typically requires an FR5 schedule of reinforcement, a comparable response is noted under an FR10 in rats trained to administer cocaine.



# Fixed ratio (FR) schedule of reinforcement (IVSA)

FR sessions enable a stable response to be obtained.

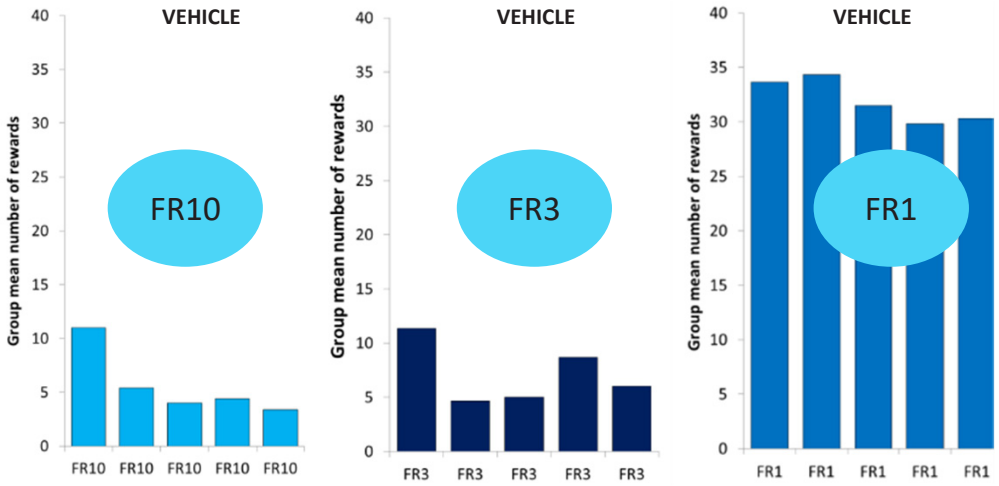
The final FR is used throughout, for final training sessions, extinction, substitution and reacquisition.

An FR that is too low may result in elevated pressing due to incidental lever pressing.

Percentage of correct responses is harder to achieve.

Extinction may take longer to achieve.

Increase the risk of overdose or satiation due to quicker succession of administrations.




An FR that is too high may result in few administrations due to the effort required to obtain the drug.

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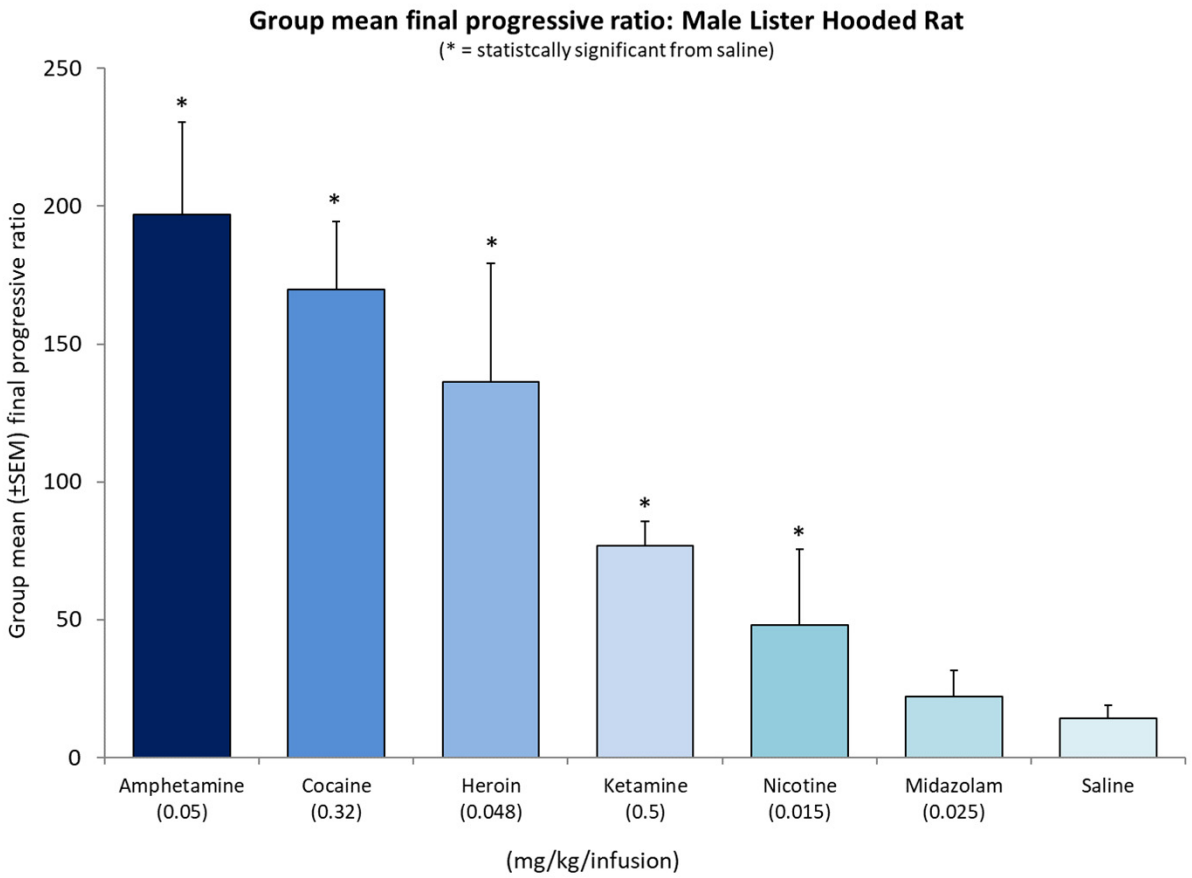
# Progressive ratio (breaking point) – (IVSA)

Provides an indication of the effort an animal will exert to obtain a single administration of the drug.

The number of lever presses required to obtain a single administration is progressively increased systematically upon each administration of a 'reward'.

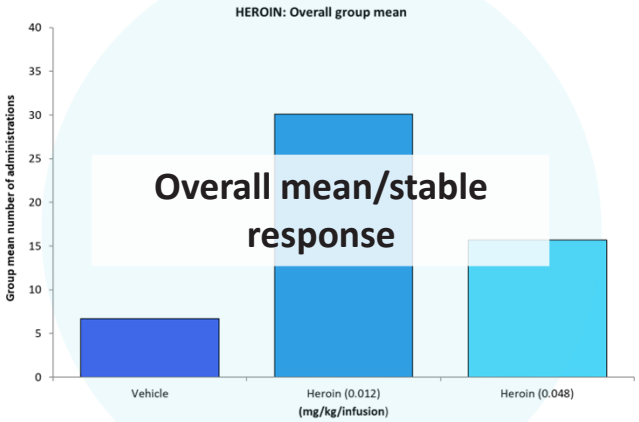
PR schedule = 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, 737, 901, 1102, 1347, 1647, 2012

Session duration typically up to 6 hours.

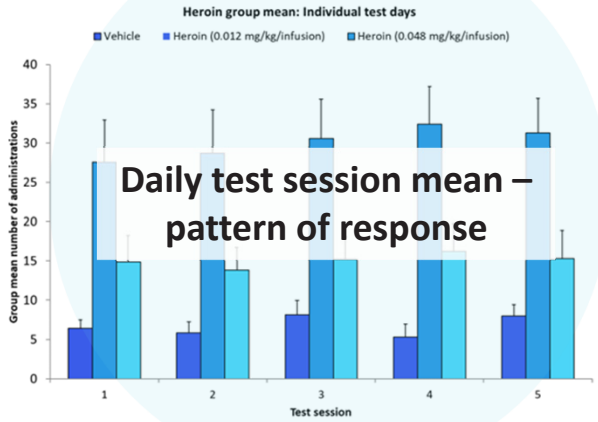


# Data interpretation (IVSA)

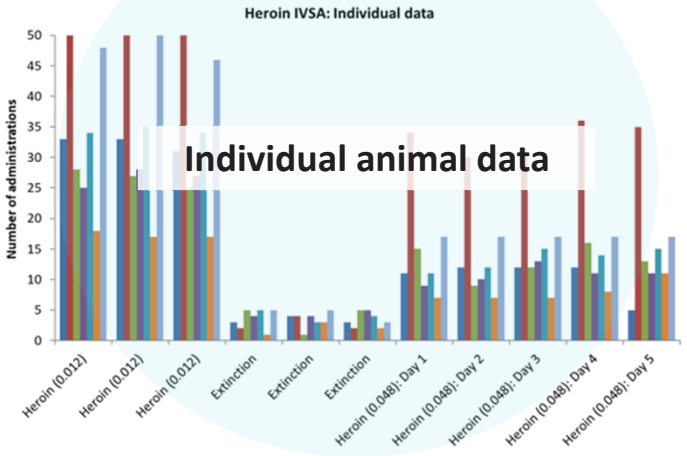
Take a holistic approach



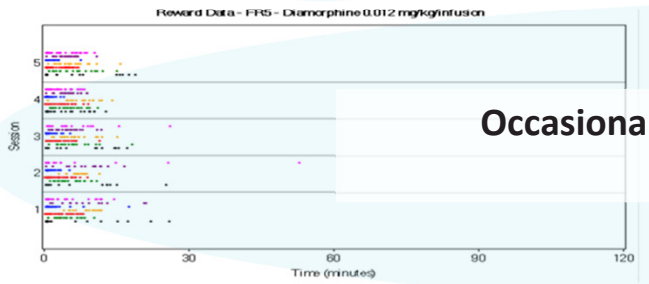

**Overall mean/stable response**



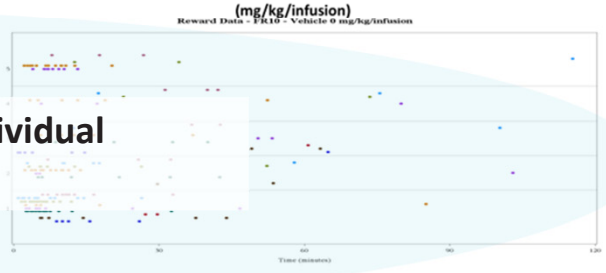

**Daily test session mean – pattern of response**




**Individual animal data**








**Occasionally may need to look at the time between individual administrations**

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# Drug discrimination (DD)

# General considerations (DD)

| ANIMALS | TRAINING DRUGS | DOSE LEVELS |
| --- | --- | --- |
| Body weights | Selected based on the pharmacology of the test article | Equate to, and multiples of the highest clinical therapeutic $C_{max}$ |
| Minimise the introduction of new staff | Scheduled under the CSA | Consider in conjunction with PK and behavioural data |
| Acclimatize to handling procedures required for dosing | Should not impair lever pressing ability | Ensure adequate washout between generalization doses |
| Can be paired/group housed, even when on a mildly restricted diet | Should not produce adverse effects | Typically, the clinical route of administration |
| | Some drugs may impair lever pressing ability | Should not impair lever pressing ability |
| | Availability, e.g., cannabinoids | |
| | Different administration routes do not affect the discriminative cue | |

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# Training confirmation (DD)

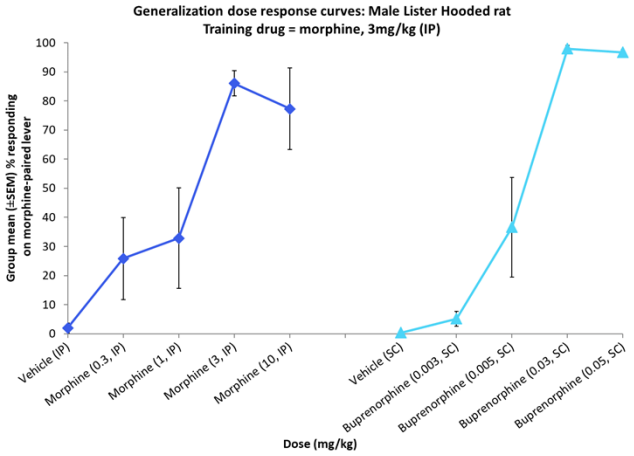
Only proceed with generalization test sessions if animals can reliably discriminate between drug and no-drug, as demonstrated by selecting the correctly paired lever for the first reward

If the incorrect lever is selected, this could imply that the animals are not responding to a discriminative cue, but are lever switching

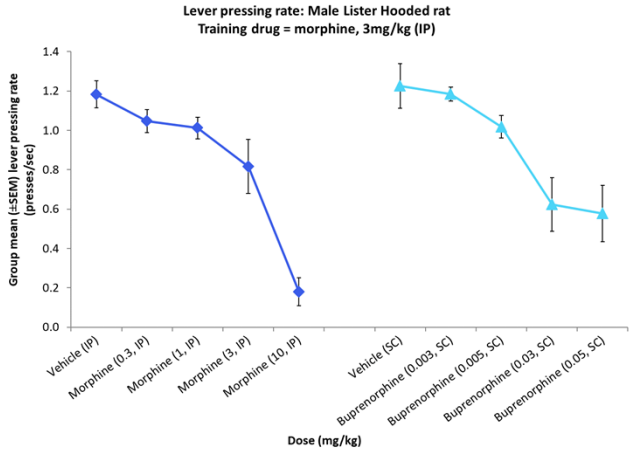
Important to perform this step if a discriminative cue has not been previously reported for the training drug

# Generalization parameters of interest (DD)

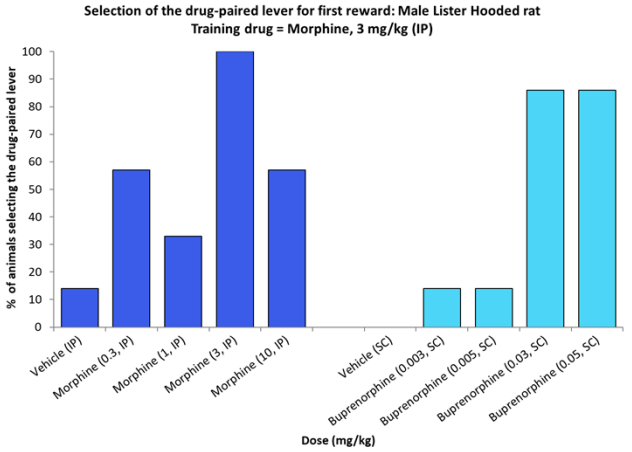
Percentage of drug-paired lever presses




Lever pressing rate (presses/second)

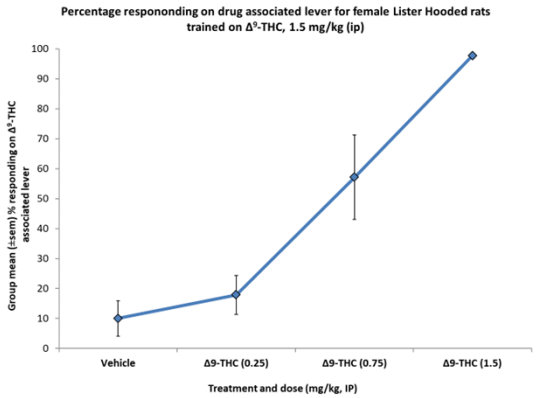



Lever providing first reward (no reinforcement)



# First lever selected – reward available (DD)

| Treatment and Dose (mg/kg, IP) | Animal ID | % Presses on Drug-Associated Lever | Lever Selected for First Reward | Lever Switches from first lever selected |
|---|---|---|---|---|
| Vehicle (0) | R0002 | 0 | ND | 0 |
| | R0005 | 19 | ND | 4 occasions |
| | R0004 | 1 | ND | 0 |
| | R0009 | 4 | ND | 0 |
| | R0003 | 3 | ND | 0 |
| | R0007 | 1 | ND | 0 |
| | R0006 | 42 | D | 2 occasions |
| $\Delta^9$-THC (0.25) | R0002 | 14 | ND | 2 occasions |
| | R0005 | 21 | D | 3 occasions |
| | R0004 | 51 | D | 2 occasions |
| | R0009 | 17 | ND | 2 occasions |
| | R0003 | 22 | D | 1 occasion |
| | R0007 | 0 | ND | 0 |
| | R0006 | 0 | ND | 0 |
| $\Delta^9$-THC (0.75) | R0002 | 17 | ND | 4 occasions |
| | R0005 | 49 | D | 3 occasions |
| | R0004 | 0 | ND | 0 |
| | R0009 | 92 | D | 1 occasion |
| | R0003 | 68 | D | 3 occasions |
| | R0007 | 76 | D | 2 occasions |
| | R0006 | 98 | D | 0 |
| $\Delta^9$-THC (1.5) | R0002 | 100 | D | 0 |
| | R0005 | 99 | D | 0 |
| | R0004 | 100 | D | 0 |
| | R0009 | 100 | D | 0 |
| | R0003 | 95 | D | 0 |
| | R0007 | 96 | D | 2 occasions |
| | R0006 | 94 | D | 0 |




The availability of a reward during generalization test sessions does not result in animals remaining on the lever selected to provide the first reward.

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# Physical dependence (PD)

# General considerations (PD)

| ANIMALS | OBSERVATIONS | DOSE LEVELS |
| --- | --- | --- |
| Typically, the strain of rat used within general toxicology studies | Performed throughout the dosing and withdrawal phase | Equate to and multiples of the highest clinical therapeutic $C_{max}$ |
| | Withdrawal phase to be of sufficient duration (at least 5 half-lives) | Ensure adequate daily dosing regimen based on PK of the test article |
| | Where possible, the same person should perform the qualitative observations throughout the study | Typically, the clinical route of administration |
| | Staff are required to be highly skilled in behavioral observations | Consider in conjunction with PK data |
| | Behaviors seen in untreated animals may be abnormal when seen at greater frequencies in treated animals | |
| | Conducted in cage, arena, hand | |

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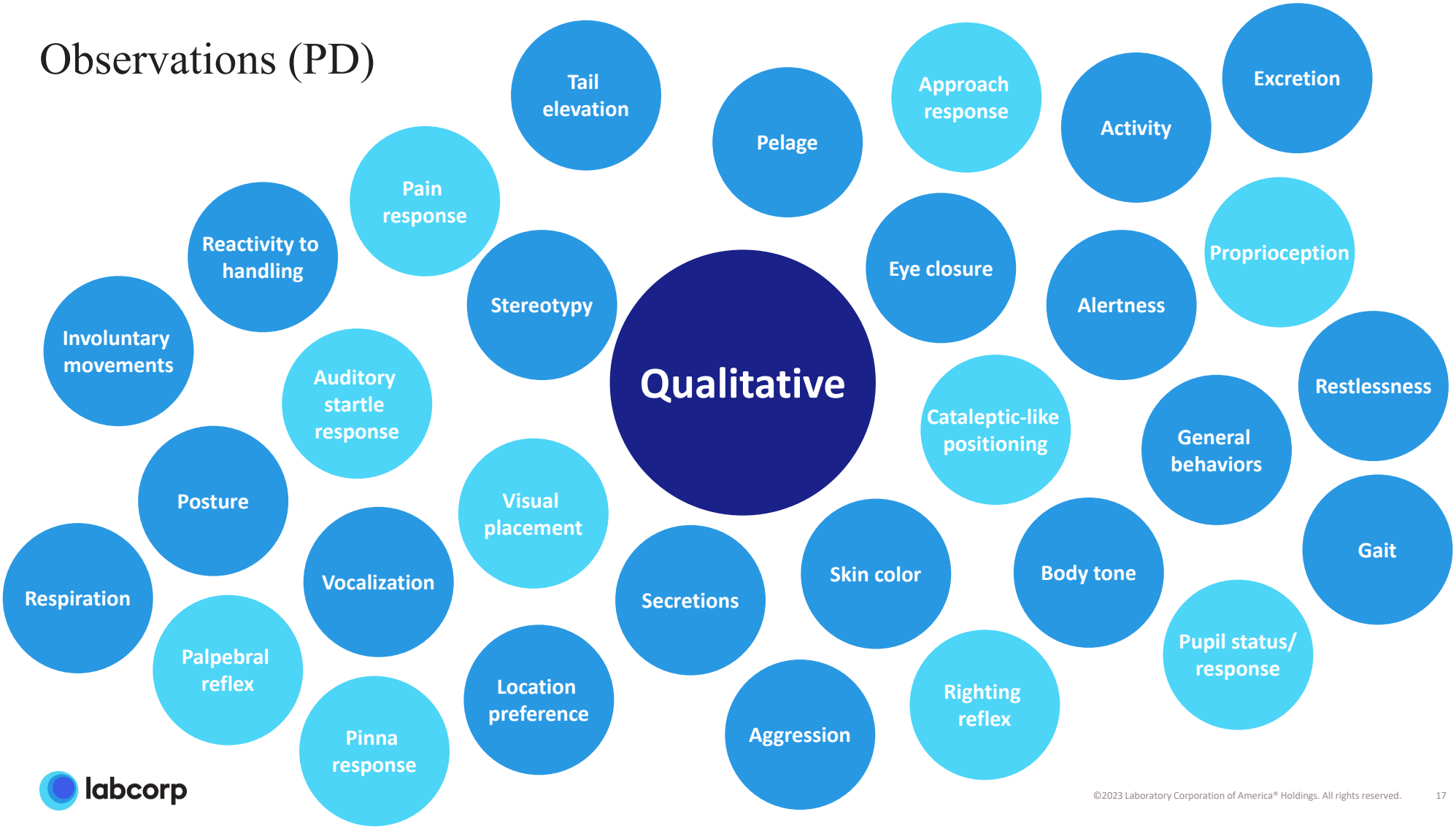
# Observations (PD)



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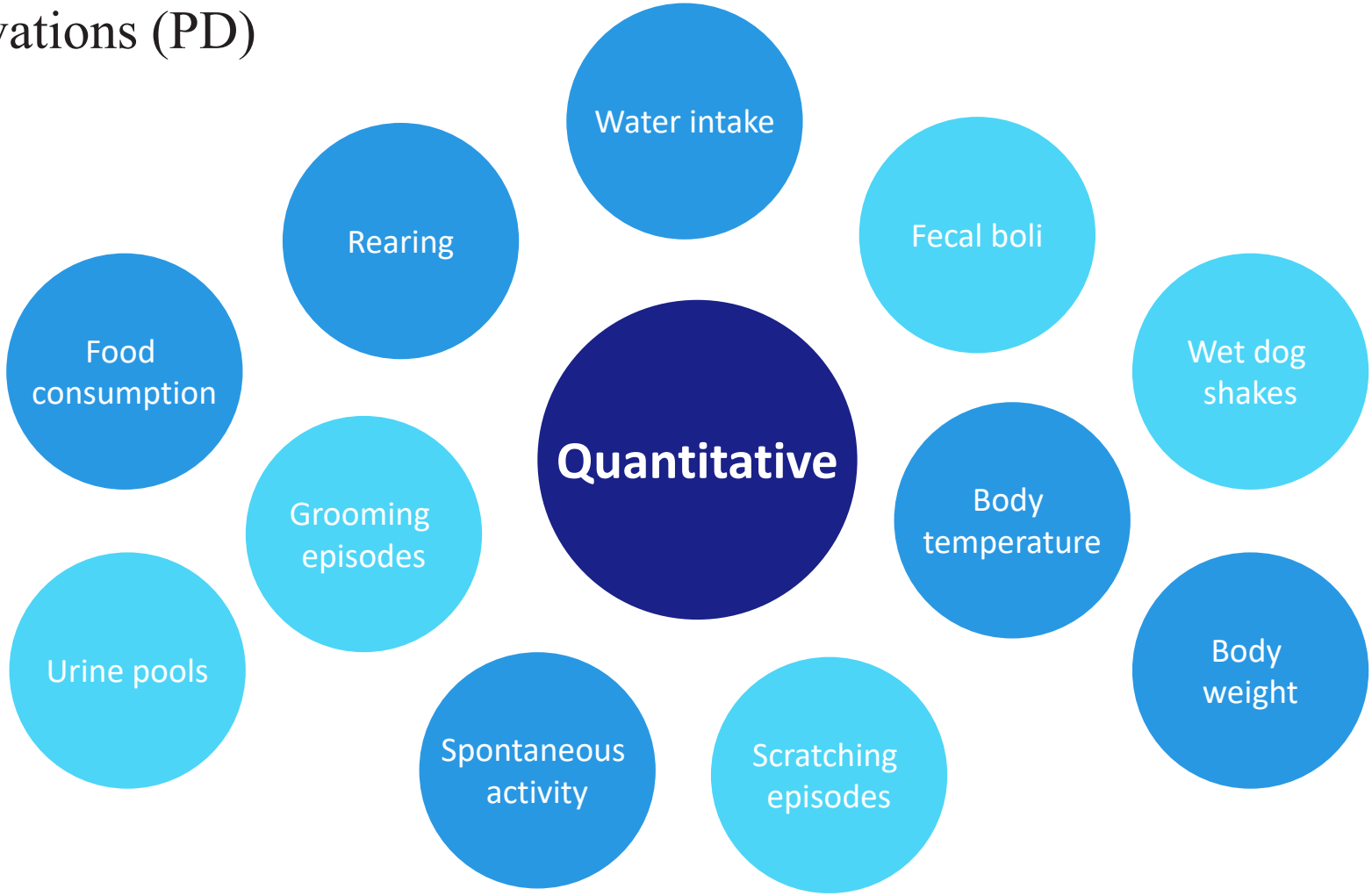


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# Comparator vs. positive control

| COMPARATOR DRUG | POSITIVE CONTROL DRUG |
| --- | --- |
| Similar class and therapeutic indication as the test article | May be a different class and therapeutic indication to that of the test article (e.g., stimulant for IVSA, opioid for physical dependence) |
| Used to compare the test article with existing treatments in terms of efficacy and/or safety | Used to confirm that the parameter(s) of interest can be reliably detected if present |
| May or may not produce an effect on the end-points under investigation | Anticipated to produce a marked/statistically significant effect on the end-points under investigation |
| Do not necessarily validate an experiment | Validates an experiment |
| In-house background data may not be readily available | In-house background data are available. Often used for model validation |

# Thank you