Protocol No: 852

THE UNIVERSITY OF WAIKATO

APPLICATION TO THE ANIMAL ETHICS COMMITTEE FOR APPROVAL OF EXPERIMENTS ON ANIMALS

ANIMAL SPECIES: Possum (Use common name) **NUMBER OF ANIMALS: 6**

STARTING DATE: 23/03/2012 **COMPLETION DATE: 31/04/2013**

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1. (a) **Name of applicant:** Chris Stanley

   (b) **Position:** Masters student

   (c) **Department: /Address for Mailing)** Psychology

   (d) **Contact Phone number & email address:** 027 319 5144 cdstanley108@gmail.com

   (e) **Qualifications and Experience:** BA (PSYC/ENGL)

   (f) Have you previously carried out related experiments? **No**

   Previous Protocol No(s)

   Applicants should attach a short report on the results of the previous experiment(s)

   (g) **Other Personnel involved** (including titles and roles):
   Dr Lewis Bizo (Senior Lecturer – Principle Supervisor)
   Dr James McEwan (Lecturer – Supervisor)
   Jenny Chandler (Animal technician)
   Kristie Cameron, Kathleen Doolan, PhD students

2. **Title of Project:** Timing in Possums: Accounting for resurgence in the Peak Procedure.

3. **Aim of Project** (written in terms that people with a non-scientific background will understand):

   The aim of this project is to test certain variables used in the peak procedure; a method developed and used by Roberts (1981) to measure the internal clock of animals. It will test if the shape of the timing function is predicted by the predictability of the end of the trial, and if alternative sources of food improve time perception on the peak procedure.

4. **Significance of this Project** (written in terms that people with a non-scientific background will understand):
   While there are explanations as to why resurgence occurs on the peak procedure (Church et al., 1991, Sanabria & Killeen, 2007), there has been limited research that directly tests them. This project will test existing and possible reasons as to what maintains response resurgence. It will also further two recent studies conducted by masters students of Waikato University.

5. **Is/Has this work already being/been carried out** (provide details)

   (a) In New Zealand? **Yes**
   Similar research has been conducted with possums and hens (see attached seminars).

   (b) Overseas? **No**

6. **Have alternative methods to achieving the aims that do not involve the use of animals been explored?** As timing is being studied it is necessary to test a live animal.

Please provide details.

7. **How will the results of this work be disseminated?**

The results of this experiment will form the basis of a thesis for a Masters degree and may be presented at national or international research conferences and may also be submitted for publication in international peer reviewed journals.

8. **Description of Experiments**

All experiments should take into account the statutory responsibility to adhere to the three important principles governing the use of animals in research, testing and teaching:

a) Refinement (refinement of procedures applied to decrease to the minimum practicable extent the negative impacts they have on the animals):
b) Reduction (reduction in the numbers of those sentient animals to the minimum necessary to achieve the scientific objective):
c) Replacement (replacement of animals with non-sentient animals or non-animal alternatives):

(a) Full details of procedures

Animals: 6 Possums.
The possums body weight will be maintained at a certain level so that they will still be motivated to work for food. On the days when animals are exposed to experimental sessions the possums will receive food as a consequence of responding on levers during the experiment that will also be supplemented with a portion of food (typically apple or carrot and dock leaves). On the days that the possum will not be working on the experiment they will receive special possum pellets in order to replace the food they would have received in an experimental session.
The possums will be weighed weekly and, if required, their post feed amounts will be changed to keep the possums at a stable body weight. The possums are maintained on a reverse day/night cycle.

Apparatus:

Throughout the duration of the experiment the possums will remain in individual cages with continuous access to water. Their housing contains a nest box at the top of their wire cage as well as an open area down below. An intelligence panel containing three response levers as well as a light above the levers will be mounted on the front wall of the cage. A diagram of the cage is shown below:

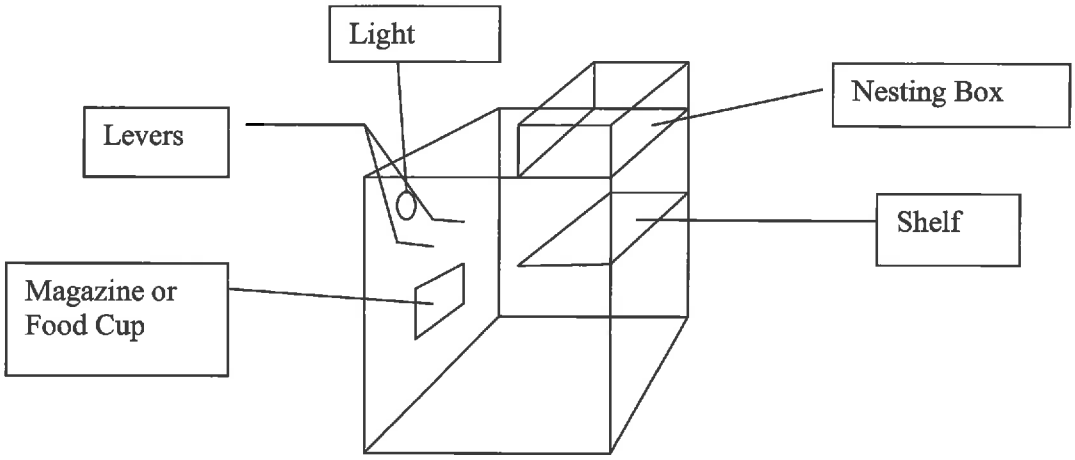


Figure 1- Possums Cage

The Peak Procedure and Resurgence

This project will be based around a method called the Peak Procedure. The peak procedure was developed by Roberts (1981) to measure an animal's ability to time. In his experiment, rats were trained on to press a lever for food on a fixed interval (FI) schedule of 40sec. Rats were rewarded with food on the first lever press following 40 seconds and Roberts named these 'food' trials. 'Empty' trials, where lever presses following 40sec were not rewarded, were then randomly mixed with 'food' trials. They lasted an average of 160sec (4 times longer than food trials). The results, which are typical of the peak procedure, are shown below.

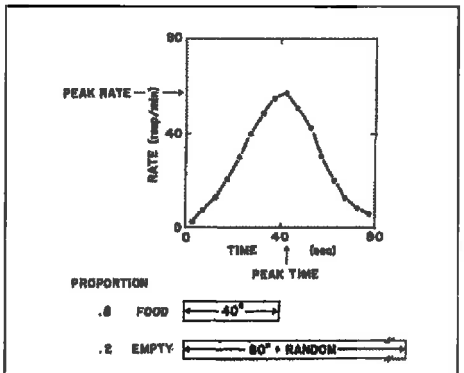
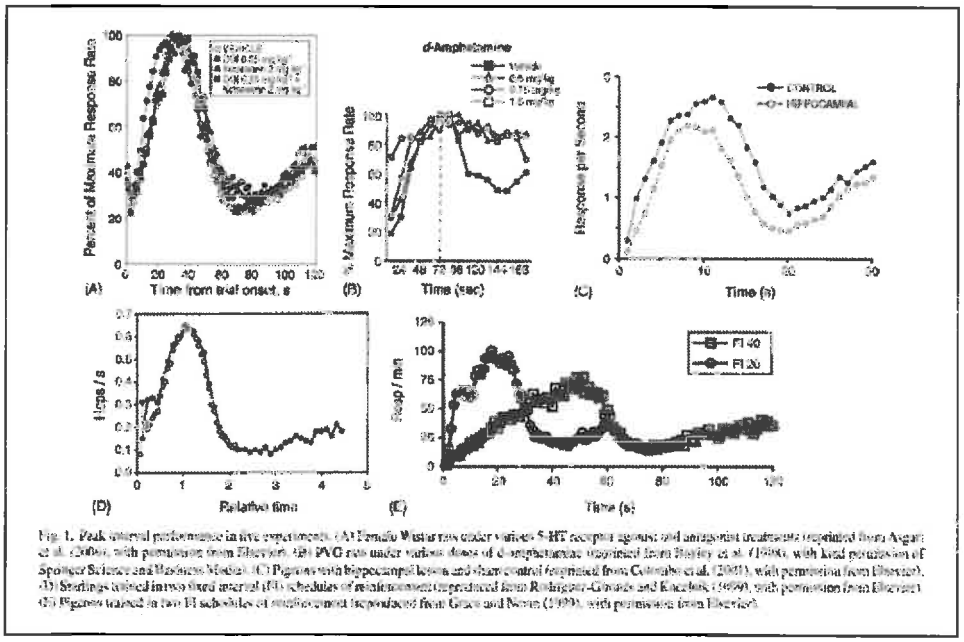


Figure 1. **The basic elements of the peak procedure and some typical results. (The results shown are from the Sec 40 condition of Part 1 of Experiment 1.)**

Roberts (1981)

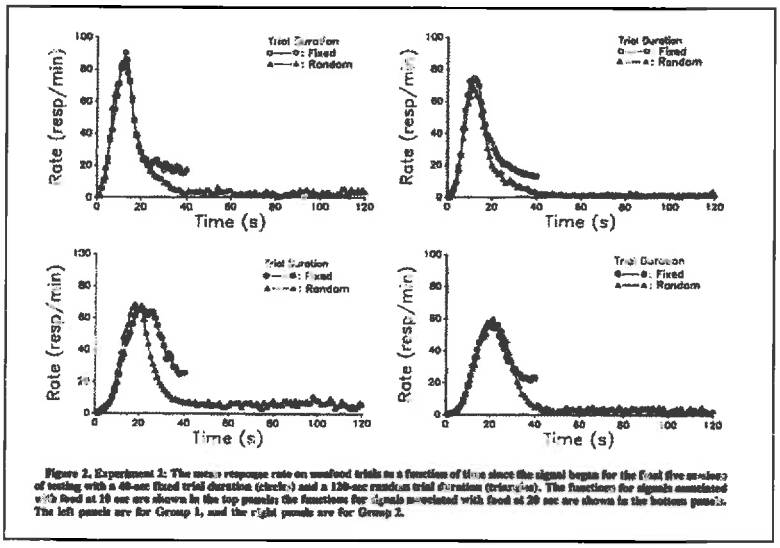
The peak occurs around the time the animals were reinforced (40sec). The steeper the curve of the peak, the more accurate an animal is at timing and vice versa.
Further study of the peak procedure found that responding started to resurge later into the empty (extinction) trials. Where only the initial peak is displayed in Roberts' results above, data collected by Sanabria and Killeen (2007) show resurgence from a variety of studies (Graphs below). Resurgence is shown by the tail following the initial peak.



Sanabria & Killeen (2007)

Church et al. (1991) state that one reason for resurgence is due to an animal's prediction of the end of the longer *empty* trial and initiation of the next *food* trial. By varying the length of *empty* trials, they were able to confuse the rat's ability to predict the end of the trial, eliminating response resurgence. Their results are displayed below.

Updated: May 2011



Church et al. (1991)

Procedures for this Project:

This project will begin by training possums to respond on a range of fixed interval (FI) schedules (scheduled from FI-10s to FI-60s). The first response after a given interval will be reinforced. Each session will contain a maximum of 100 trials. Once responding is reliably occurring at a maximum near the time of reinforcement, the peak procedure will be introduced. FI trials will be randomly mixed with *empty* trials lasting three or four times as long. Once responding typical of the peak procedure is observed, 3 new conditions will be introduced. The first will be based on the Church et al. (1991) paper (mentioned above). The length of empty trials will vary in order to confuse the possum's ability to accurately predict the end of the trial.

An alternative reason, and hypothesis of this project, is that resurgence occurs because there is no other behaviour for animals to engage in following the delivery of reinforcement. The second condition will present possums with two schedules of reinforcement running concurrently. A FI/peak procedure will be presented on one lever, while a second variable interval (VI) schedule will be presented on the second. The VI lever will be made contingent on a response after a given time on the FI schedule. Put simply, the possums will be reinforced for other behaviour while waiting for the peak procedure trial to end. The hypothesized result being that response resurgence should be nonexistent and that timing performance will be improved.

References:

Church, R. M., Miller, K. D., Meck, W. H., &Gibbon, J. (1991). Symmetrical and asymmetrical sources of variance in temporal generalization. *Animal Learning & Behavior, 19*, 207–214.

Roberts. S. (1981). Isolation of an Internal Clock. *Journal of Experimental Psychology: Animal Behavior Processes, 7*, 3, 242-268.

Sanabria. F., & Killeen. P. R. (2007). Temporal generalization accounts for response resurgence in the peak procedure. *Behavioural Processes, 74*, 126-141.

(b) The statistical design of the experiments

This project will use a within subject design. The results obtained from the subjects will be compared using parametric statistics such as one way ANOVA.

9. List the relevant SOP's (number and full title) to be used:

10. (a) **Where experiments will be conducted:** Psychology Animal Behaviour Laboratory, No 3 Dairy

(b) **Where the animals will be housed:** Individually in cages for the duration of the experiment.

(c) **Person in immediate charge of laboratory and housing:** Jenny Chandler

(d) **Veterinary advisor to the laboratory:** Ali Cullum

11. **Is there an operational procedure required for the use of a product (drug/chemical) in these experiments? No**

If 'Yes' this will require an Institutional Drug Administration Order, this should be arranged with the Institutional Operating Plan Validator.

See Appendix 1: ***Is an Institutional Drug Administration Order Required?*** **Yes/No**

Name the product: _____

_____

12. (a) **Anaesthetic:**

Local: _____

General: _____

(b) **Method of Restraint:** _____

(c) **Will the animal have to recover from anaesthetic?** _____

(d) **How will you deal with post-operative pain and/or discomfort?** _____

_____

_____

13. **What is the fate of the animals at termination of experiment?** Retained in experimental laboratory.

14. **Has this application in whole or in part previously been declined approval by another Animal Ethics Committee?** **No**

15. **For experiments to be undertaken at Ruakura or at other facilities under the control of another Animal Ethics Committee, has an application also been made to that Committee:** **No**
If 'YES' state which Committee _____

16. **Is any of this work being used in a thesis to be submitted for a degree at The University of Waikato?** **Yes**

17. **Are any permits (e.g. DOC) or approvals (e.g. Iwi) required?** **No**

If 'YES':
Have the permits or approvals been obtained? **Yes/No**

List details of permits/approvals required _____

18. **I have read and understand the conditions outlined in the Code of Ethical Conduct for the Use of Animals for Teaching and Research.** **Yes**
http://www.waikato.ac.nz/research/unilink/uow_only/Approved%20Code%202010%20-%202014.pdf

19. **I have read the Good Practice Guide for the Use of Animals in Research, Testing and Teaching**
http://www.biosecurity.govt.nz/files/regs/animal-welfare/pubs/naeac/guide-for-animals-use.pdf

Yes

20. **Further conditions:**
If this application is approved, I will inform the Committee of any changes in the project or unexpected outcomes affecting animal welfare, and any event (beyond any approved manipulation) impacting adversely on animal welfare.

Signed by the applicant: _____ **Date:** 9/3/2012

I accept responsibility for this project's compliance with the University's Code of Ethical Conduct for the Use of Animals for Teaching and Research.

Signed by the Supervisor: _____ **Date:** 9/3/2012.

I accept responsibility for this project's compliance with the University's Code of Ethical Conduct for the Use of Animals for Teaching and Research.

Approved/NOT approved

Signed on behalf of the Committee: _____
(Chairperson)

Date: 16/03/12

Determinants of Timing Performance on the Peak Procedure

THE UNIVERSITY OF WAIKATO
Te Whare Wānanga o Waikato

Learning Behaviour Welfare Research Unit

Mark E. McHugh, T. Mary Foster, & Lewis A. Bizo
The University of Waikato

Aims

- To investigate the timing abilities of domestic hens (*Gallus Gallus Domesticus*).
- To investigate the effect of excessively long extinction trials on peak procedure performance.
- Investigating the development of resonance patterns during the longer extinction trials, as reported by Kirkpatrick-Steger et al (1996) as shown in figure 1.

General Method

Subjects:

- 6 domestic hens (*Gallus Gallus Domesticus*)

Apparatus:

- Operant chamber with a central key and central food magazine.
- Reinforcer 2.5-s access wheat

Procedure:

- Trained to respond on a FI20-s and FI40-s schedules
- 2.5s access to reinforcement and 5-s ITI
- Peak procedure has a 4:1 mix of FI and Peak Interval (PI) trials.
- PI trials 10 times longer than the FI trials.
- PI trials are run in extinction.

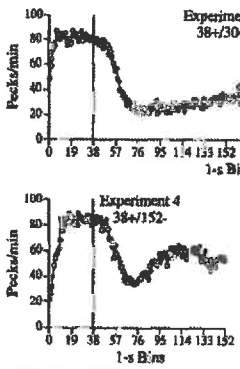


Figure 1: Successive 1-s bins of last 6 day of peak testing. (Kirpatrick-steger et al, 1996)

Condition 1 results:

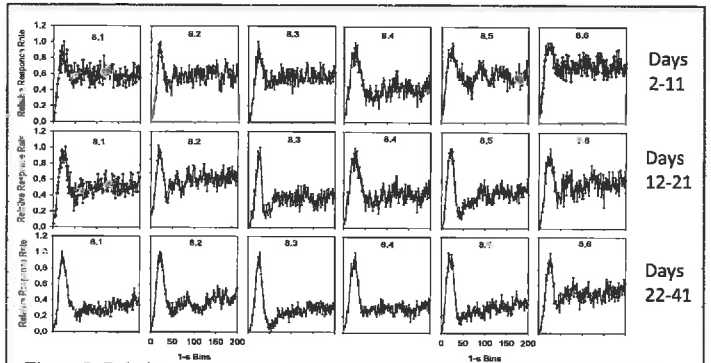


Figure 2: Relative response rate verse time day 2-11, 12-21 and 22-41 days on peak testing

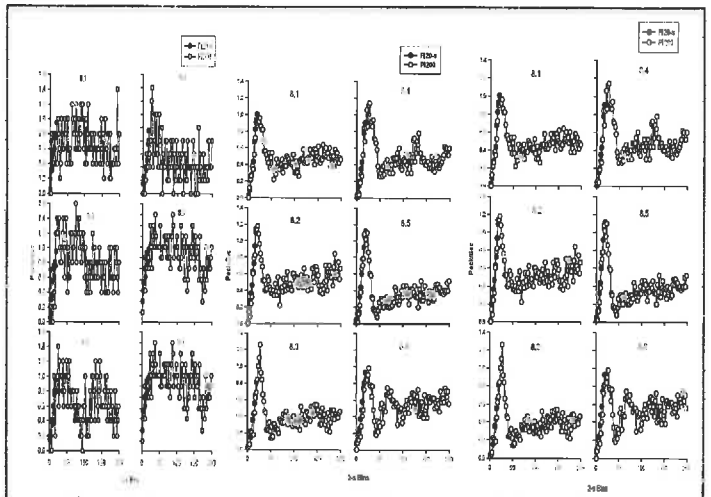


Figure 3: Average response rate verse time, days 1, 12-21 and 22-41 days on peak testing and FI20-s trials

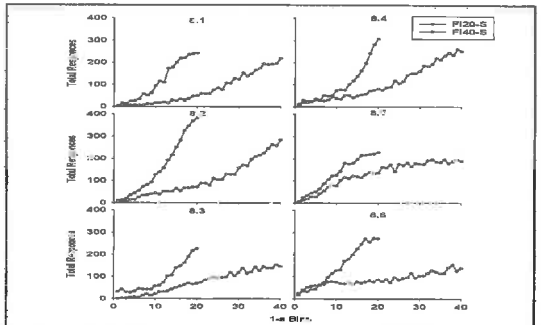


Figure 4: Total responses on last five days of FI20-s training and total responses days 10-15 of FI40-s training

Condition 1

- FI20-s pre-training (30 sessions).
- PI trials 10 times FI duration. (PI200-s)
- No response required to end the PI trial.
- 41 sessions of peak testing

Condition 2

- FI40-s pre-training (Formation of FI Scallop)
- PI trials 10 times FI duration (PI400-s)
- No response required to end PI trials
- Subsequent conditions will decrease duration of the extinction trials.

References

Kirkpatrick-Steger, K., Miller, S.S., Betti, C.A.,Wasserman, E.A.(1996). Cyclic responding by pigeons on the peak timing procedure. *Journal of Experimental Psychology: Animal Behavior Processes*, 22, 447–460.

Sanabria, F., & Killeen, P.R. (2007). Temporal generalization accounts for response resurgence in the peak procedure. *Behavioural Processes*, 74, 126-141.

Contact: mem17@waikato.ac.nz

Peak Procedure Performance in the Common Brushtail Possum (*Trichosurus vulpecula*)

Rachael A. Lockhart, James S. McEwan, J., & Lewis A. Bizo
University of Waikato, Hamilton, New Zealand

THE UNIVERSITY OF WAIKATO
Te Whare Wānanga o Waikato

Learning Behaviour Welfare Research Unit

Aims

- To investigate timing performance in the common brushtail possum.
- To investigate effect of a response requirement on peak procedure performance.
- To investigate response resurgence during "short" vs. "long" extinction trials

General Method

Subjects:

- 3 male and 2 female adult common brushtail possums (*Trichosurus vulpecula*).

Apparatus:

- Live in chambers with 3 levers and central food magazine.
- Reinforcer 3-s access to cocoa puffs & flaked wheat.

Procedure:

- Trained to lever press on a series of Fixed Interval (FI) schedules, FI 15-s, FI 30-s, and FI 60-s.
- Peak procedure trials a 4:1 mix of FI and Peak Interval (PI) trials.
- PI trials are run in extinction.

Experiment 1

- FI 15-s, FI 30-s & FI 60-s (30 sessions per condition).
- PI trials 3 times FI duration.
- Response required to end the PI trial.
- 30 sessions on peak procedure

Experiment 2

- FI 30-s, and then 4:1 mix of FI and PI trials.
- PI trials 3 or 10 times FI duration.
- No response required to end the PI trials.
- 28 sessions on peak procedure

Email: lbizo@waikato.ac.nz

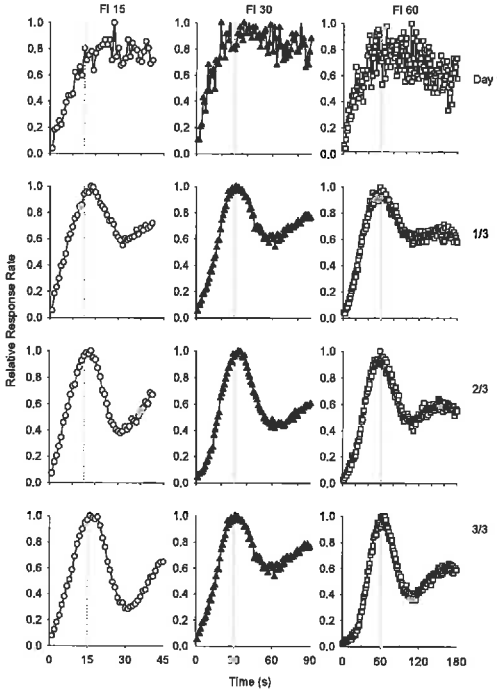


Fig 1. Relative response rate versus time for the 1st day, and successive 3rds of training on the peak procedure in Expt 1.

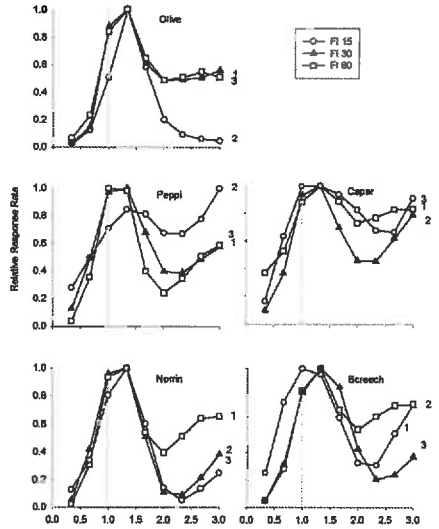


Fig 2. Relative response rate versus relative time for last 5 days of each FI schedule for each animal in Expt 1.

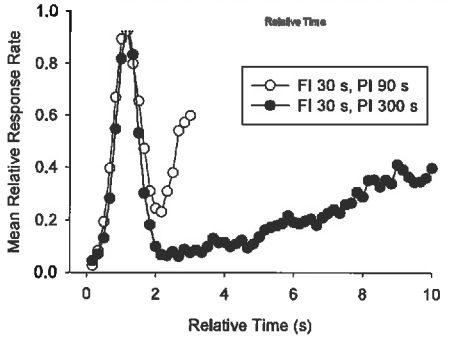


Fig 3. Mean relative response rate versus relative time for Expt 2.

Conclusions

- Reasonable superposition up to twice the FI duration.
- A response requirement to end the PI trials promoted responding at the end of the PI trial.
- Removal of response requirement did not remove responding at end of the peak interval trials.
- No evidence of multiple peaks on longer trials (as per Kirkpatrick-Steger et al., 1996).
- Resurgence shown by a montonic increase in responding after the initial peak towards the end of the peak interval trials (as per Sanabria & Killeen, 2007)

References

Kirkpatrick-Steger, K., Miller, S.S., Betti, C.A.,Wasserman, E.A.(1996). Cyclic responding by pigeons on the peak timing procedure. *Journal of Experimental Psychology: Animal Behavior Processes*, 22, 447–460.

Sanabria, F., & Killeen, P.R. (2007). Temporal generalization accounts for response resurgence in the peak procedure. *Behavioural Processes*, 74, 126-141.

ANIMAL USE STATISTICS
APPLICATION/FINAL RETURN FORM

Protocol ID
852

If more than one animal type is required then fill in one form for each type

Application: When applying to the AEC for approval of a manipulation the applicant should complete Box 1 and **enter in Questions 2 - 9, in the *'Planned'* column (P)**, the appropriate figures for the number of animals required.

Final return: When the manipulation is completed the approved original application form will be returned. Boxes 2 to 10 should then be completed in the *'Used'* column (U) by entering appropriate figures for the number of animals which were actually used.

NAME/INSTITUTION:

1. Animal type Possum_____ Code: t_____
(see Appendix A page 3 of this form)

2. Source of animals (number)

		P	U
Breeding unit	a		
Commercial	b		
Farm	c		
Born during project	d		
Captured	e	6	
Imported	f		
Public sources	g		
TOTAL = A			

3. Status of animals (number)

		P	U
Normal/conventional	a	6	
*SPF/germ free	b		
Diseased	c		
Transgenic/chimaera	d		
Protected species	e		
Unborn/prehatched	f		
Other	g		
TOTAL			

**Specific pathogen free*

4. Main category of manipulation/use (enter the total from 2 above in one box only)

		P	U			P	U			P	U
Teaching	a			Animal husbandry	d			Veterinary research	g		
Species conservation	b			Basic biological research	e	6		Testing Development of alternatives	h i		
Environmental management	c			Medical research	f			Other	j		

5. Any re-use of animals (number to be inserted)

		P	U			P	U	
No prior use	a			Previously used	b	6		Total a + b =

6. Grading of manipulations/use (number in each grade to be inserted). For examples of grades of manipulation see these in "Grades of manipulation" Appendix B on page 3 of this form.	Grade	P	U
Manipulations that are expected to cause no impact or virtually no impact. **No impact**	A		
Manipulations of minor impact and short duration. **Little impact**	B	B	
Manipulations of minor impact and long duration or moderate impact and short duration. **Moderate impact**	C	6	
Manipulations of moderate impact and long duration or high impact and short duration. **High impact**	D		
Manipulations of high impact and long duration. **Very high inpact**	E		

7. Expected date of completion: 31/04/2013

ANIMAL DISPOSITION/FATE AT CONCLUSION OF EXPERIMENT/TEACHING EXERCISE ETC OUTLINED IN THIS PROTOCOL

8. ALIVE		P	U	**9. DEAD**		P	U
Retained by your institution's	a	6		Killed for dissection, sampling, taking organs	a		
Returned to commercial farmers	b			Died/destroyed in the course of the manipulation/use	b		
Released to the wild	c			Euthanased after manipulation or use	c		
Disposed of to others	d			Died/destroyed for reason not associated with manipulation/use	d		
TOTAL ALIVE	=B=			TOTAL DEAD	=C=		

To be completed at conclusion of protocol

10. GRAND TOTAL MANIPULATED/USED = B + C

Check on the final return that B + C = A in the "Used" column of Box 2.

Appendix A

ANIMAL TYPE CODES:
Type of animal used. No distinctions on basis of sex, age, breed, strain or physiological condition.

BOX 1	CODE LETTERS				CODE LETTERS		
Rodents	1	a	Mice	Birds	1	p	Fowls, Chickens
	1	b	Rats		1	q	Pigeons
	1	c	Guinea Pigs		1	r	Other Birds
	1	d	Hamsters	Miscellaneous	1	s	Marine Mammals
Rabbits	1	e	Rabbits		1	t	Possums
Farm Animals	1	f	Sheep		1	u	Reptiles
	1	g	Cattle		1	w	Amphibia
	1	h	Goats		1	x	Fish
	1	j	Deer	Other	1	y	Other Species (°name)
	1	k	Pigs				
Other Domestic	1	m	Horses				
Mammals	1	n	Dogs				
	1	o	Cats				

Appendix B

Grading of Manipulation Examples

Grade A – "No impact or virtually no impact"

Examples:

Mental state: Field observations of grazing behaviour on farms, or benign handling of tame and trained animals that are familiar with all personnel and procedures and with the place where the procedures are conducted.

Food/water: Animals kept outdoors eating their usual food in appropriate amounts; grazing trials on treated pastures; offering supplements to naturally available food; provision of complete, balanced rations to meet all nutritional requirements of animals maintained indoors.

Environmental challenge: Exposure to ambient conditions that are within the thermoneutral range; reduced barometric pressures which do not cause increases in red blood cell production.

Disease/injury/functional impairment: Studies of healthy uninjured animals that are kept in physical conditions which do not themselves lead to injuries such as lameness or compression sores; studies to establish normal characteristics of healthy animals.

Behaviour: Studies of wild or undomesticated animals in their natural habitats; field studies of domesticated animals.

Grade B – "Little impact"

Manipulations of minor impact and short duration

Examples:

Mental state: Experiments on completely anaesthetised animals that do not regain consciousness; simple venipuncture or venisection; injection of non-toxic substances; skin tests which cause low- level irritation without ulceration/erosion; feeding trained animals by orogastric tube; movement of free-range domesticated animals to unfamiliar housing; minor restrictions of water and/or feed intake beyond the normal period of satiation.

Food/water: Water priming for kidney function tests; short-term overall food intake restrictions or excesses that are within usual tolerance levels for the species; short-term changes in dietary composition that cause no clinical signs of deficiency or toxicity, but which would cause such symptoms in the longer term.

Environmental challenge: Exposure to levels of cold or heat that are outside the thermoneutral range, or barometric pressures that increase red blood cell production, but which remain within the capacity of the animals to adapt and do not lead to debility in the long term.

Disease/injury/functional impairment: Studies of vaccines using killed pathogens; tuberculosis tests; induction of mild fever without other debilitating effects; induction of subclinical parasitism; healing of minor superficial incisions, cuts or wounds; minor surgical and/or pharmacological modification of homeostatic capacity (for example, creation of non-obstructive gut fistulae; splenectomy; endocrine gland removal with complete and permanent hormone replacement therapy); physical conditions which cause transient lameness of low intensity, mild compression sores or abrasions.

Behaviour: Mild and short-term physical restraint; keeping free-range domesticated animals in a yard; movement of free-range domesticated livestock to unfamiliar housing; operant conditioning with positive reinforcement in barren laboratory environments; benign preference tests in unnatural surroundings.

Grade C – "Moderate impact"

Manipulations of minor impact and long duration or moderate impact and short duration

Examples:
Mental state: Recovery from major surgeries like thoracotomy, orthopaedic procedures, hysterectomy or gall bladder removal with effective use of analgesics; surgical procedures on conscious animals but with the use of local anaesthesia and systemic analgesic; movement of excitable free-range domesticated livestock to unfamiliar housing; short term capture, handling and restraint of wild or semi-domesticated animals that exhibit marked flight responses; moderate restrictions of water and/or feed intake beyond the normal period of satiation.
Food/water: Simulation of usual overall intake restrictions often experienced by pregnant/lactating ruminants during cold winters or drought; dietary induction of milk fever in cattle; induction of mild deficiency or toxicity signs by feeding diets containing inadequate or excessive amounts of essential nutrients.
Environmental challenge: Short-term exposure to severe extremes of cold or heat which would lead to collapse if prolonged.
Disease/injury/functional impairment: Studies of live vaccines; induction of clinical parasitism; induction of mild reversible infectious diarrhoea; moderate surgical and/or pharmacological modification to homeostatic capacity (for example, limited gut resection; endocrine gland removal with delayed or incomplete hormone replacement therapy); physical conditions that cause minor chronic lameness or other injuries; studies of the effects of infectious or toxic agents that cause rapid death without distress.
Behaviour: Medium-term restrictions of instinctive behaviour; medium-term holding of ruminants in a metabolism crate; long-term restraint leading to the development of reversible stereotypies; changing social group composition.

Grade D – "High impact"

Manipulations of moderate impact and long duration or high impact and short duration

Examples:
Mental state: Recovery from major surgery under anaesthesia without the use of postoperative analgesics; marked social or environmental deprivation; longer term capture, handling, restraint or housing, without the use of tranquilisers, of wild or semi-domesticated animals that exhibit marked flight responses.
Food/water: Dietary induction of advanced pregnancy toxaemia in sheep or ketosis in dairy cattle; dietary induction of advanced signs of nutrient deficiency or excess; severe deleterious effects of dietary toxins; severe restrictions of water and/or feed intake beyond the normal period of satiation.
Environmental challenge: Prolonged exposure to severe cold or heat that would lead to failure of thermoregulation and collapse, but the exposure is terminated just before those outcomes.
Disease/injury/functional impairment: Studies of severe facial eczema; induction of severe diarrhoea or severe infectious pneumonia; protracted or irreversible pharmacological modification of homeostatic capacity (for example, chemical induction of diabetes mellitus without replacement therapy); marked surgical modification of homeostatic capacity (for example, extensive gut resection; cutting of sensory or motor nerves serving large areas of the body from which no self-mutilation injury results; precise lesioning of limited areas of the brain but with intervention before collapse); physical conditions that cause moderate chronic lameness or other injuries; studies of the effects of infectious and toxic agents that cause either a protracted death with minor distress or a rapid death with moderate distress.
Behaviour: Application of marked and repeated noxious stimuli from which escape is impossible; prolonged periods (several hours or more) of close physical restraint; marked alterations to the perceptual or motor functions of animals to test consequent behaviour.

Grade E – "Very high impact"

Manipulations of high impact and long duration

Examples:
Mental state: Conducting major surgeries without the use of anaesthesia on control animals in assessing efficacy of analgesics; testing the efficacy of analgesics in animals with severe induced pain.
Food/water: Experiments that cause animals to die from poisoning by toxins in the diet; protracted and severe restrictions on water and/or feed intake.
Environmental challenge: Purposeful exposure of conscious animals to lethal extremes of cold, heat or barometric pressure which duplicate naturally occurring conditions.
Disease/injury/functional impairment: Studies of methods for killing pest animals; cutting of sensory or motor nerves serving large areas of the body from which self-mutilation injury results; evaluation of vaccines where death is the measure of failure to protect; studies of the effects of infectious or toxic agents which cause either a protracted death with marked distress or a rapid death with severe distress.
Behaviour: Application of marked and repeated extremely noxious stimuli from which escape is impossible; prolonged periods (several hours or more) of close physical restraint.