SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

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Item #	Section/Subsection/Item	Description	Check for approval
	A. General		
1.	Title of the review	A systematic review on intra-species olfactory signals and social behaviour in captive mammals.	
2.	Authors (names, affiliations, contributions)	Amanda J. Barabas: Graduate student, Department of Animal Science, Purdue University; design study, data search and analysis, writing manuscript Brianna N. Gaskill: Associate Professor, Department of Animal Science, Purdue University; design study, solve discrepancies when forming selection criteria and during search, writing manuscript.	
3.	Other contributors (names, affiliations, contributions)		
4.	Contact person + e-mail address	Amanda Barabas abarabas@purdue.edu	
5.	Funding sources/sponsors	NA	
6.	Conflicts of interest	No conflicts of interest	
7.	Date and location of protocol registration	-	
8.	Registration number (if applicable)	-	
9.	Stage of review at time of registration	The review will start after registration	
	B. Objectives		
	Background		
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	In captive animals, social stress is often a cause of poor welfare and can have far reaching consequences for human research and production. In laboratory settings, aggression in male mice reduces both physical and mental welfare, and wounding due to escalated fighting is one of the most highly reported injuries in mice [1,2]. In nonhuman primates, aggression can have a complex effect on both hormonal and other behavioural measures [3]. Ultimately, this can impact the validity and reliability of research data through unexplained data variation. In production settings, aggression among unfamiliar pigs not only reduces welfare, but presents an economic burden through increased veterinary care, poor carcass quality, and reduced growth and reproduction rates [4]. As explained below, previous work has shown that olfactory stimulation can improve general welfare in various species. Odours produced by an animal's own species (i.e.: pheromones) have been shown to reduce stress in a variety of captive settings. Feliway™, a synthetic facial pheromones from domestic cats, is commonly implemented in veterinary clinics to reduce	

		stress and is recommended for use in shelter environments [5,6]. In pigs, the pig appeasing pheromone (PAP) originates from mammary sebaceous glands and has been shown to reduce wounding from aggression and promote feeding behaviour and weight gain in newly mixed piglets [7,8]. While PAP has been shown to affect social behaviour in weanling pigs, similar treatments in other species and ages are not often used. Perhaps it is due to the quality of studies done in other species or using other odours. While PAP is considered a pheromone, it is possible that other odours or odour combinations may be beneficial. For an odour to be considered a pheromone, its behavioural effect must meet a list of five criteria [9], which includes being effective at naturally occurring concentrations and, in the case of mixtures, every component must be proven necessary to elicit the behavioural response. It is possible that effective odour treatments may not necessarily meet this definition and are excluded from treatment searches. Due to term familiarity, pheromones may be more likely to be searched. Even though other odours can still be beneficial, it is possible that terminology affects how much a treatment is found and implemented. Many options will be excluded if searches are limited to strictly pheromones. For instance, in male mice, removing cage odours is a known trigger of aggression [10]. The simple preservation of the odour profile from used nesting material is one of the few effective treatments to reduce aggression after cage cleaning [11]. Recently, it has been shown that the nest holds a complex protein mixture that contains information about individual strain, age, and reproductive status [12]. It may not be practical to determine if every component in the nest is necessary to reduce aggression. The purpose of this systematic review is to provide an overview of all intra-species odours and odour mixtures that may influence non-reproductive social behaviours in captive mammals. We will provide an overview of all studies until May 31, 2020 that measure social behaviour and utilize odour treatments that originate from their species of focus. We will also evaluate the quality of the methods in these studies and address any subsequent research gaps. Odour treatments were chosen as they could prove to be ethologically relevant and practical solutions for social stress.	
	Research question		
11.	Specify the disease/health problem of interest	Animal welfare, scientific quality	
12.	Specify the population/species studied	All terrestrial, mammalian species in a captive setting	

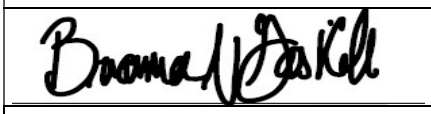
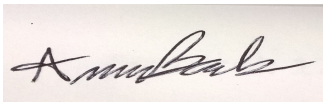
13.	Specify the intervention/exposure	Treatment with intra-species odours/gland secretions or odour preservation in the environment designated by reduced or partial cleaning schedule	
14.	Specify the control population	Matching population without odour exposure or preservation	
15.	Specify the outcome measures	Rate of non-reproductive social behaviour (aggressive, affiliative, investigative); measures of stress such as hormone levels, increased heart rate, abnormal/fear behaviour rate	
16.	State your research question (based on items 11-15)	How effectively do intra-species odours influence non-reproductive social behaviour and social stress in captive, terrestrial mammals?	
	C. Methods		
	Search and study identification		
17.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)	X MEDLINE via PubMed X Web of Science ☐SCOPUS ☐EMBASE X Other, namely: Commonwealth Agriculture Bureau; United States Department of Agriculture National Agriculture Library ☐Specific journal(s), namely:	
18.	Define electronic search strategies (*e.g.* use the step by step search guide[15] and animal search filters[20, 21])	When available, please add a supplementary file containing your search strategy: [insert file name]	
19.	Identify other sources for study identification	X Reference lists of included studies X Books ☐Reference lists of relevant reviews ☐Conference proceedings, namely: ☐Contacting authors/ organisations, namely: ☐Other, namely:	
20.	Define search strategy for these other sources	-Check each reference list of included studies for possible relevant studies not found by our search in the database. We will use the following books already in our possession: *Pheromones and Animal Behavior: Chemical Signals and Signatures* [13] *Olfaction in Animal Behaviour and Welfare* [14]	
	Study selection		
21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	1) pre-screening based on title and abstract 2) full-text screening of the eligible articles	
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	Each phase: 2 independent observers (AJB and undergrad assistant) per article. Differences will be solved through discussion or by consulting a third investigator (BNG)	
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)	Inclusion criteria: control vs experimental treatment, including repeated measure studies in which a control is the subject's baseline and the experimental measure is taken after treatment Exclusion criteria: any other study design	
24.	Type of animals/population (*e.g.* age,	Inclusion criteria: studies on captive terrestrial mammals,	

	gender, disease model)	of any sex or strain/breed. Exclusion criteria: studies that use aquatic mammals, insects, or ectotherms	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	Inclusion criteria: any study that uses odour treatments that are produced by the species of focus or synthetic equivalents Exclusion criteria: studies using odour treatments from a different species (i.e.: predator/prey odours), plant sources, or synthetic origins	
26.	Outcome measures	Inclusion criteria studies that measure rates of non-reproductive social behaviour (aggressive, affiliative, and investigative behaviours). Studies may or may not include stress measures. Exclusion criteria: studies that don't measure social behaviour; studies that measure reproductive behaviour	
27.	Language restrictions	Inclusion criteria: Studies written in English Exclusion criteria: Studies in any language other than English	
28.	Publication date restrictions	Inclusion criteria: All studies up to May 31, 2020 Exclusion criteria: Any study after May 31, 2020	
29.	Other	Inclusion criteria: Exclusion criteria: not an original study	
30.	Sort and prioritize your exclusion criteria per selection phase	Selection phase: 1. Study not in English 2. Not an original study 3. Full text not available 4. Study occurs after May 31, 2020 5. Study does not use a terrestrial mammalian species 6. Study does not use odour treatments from species of focus 7. Study does not measure non-reproductive social behaviour 8. Study does not assess the **direct impact** of the odour on social behaviour. Cause/effect relationship is not explored. 9. Study does not have a control group 10. Duplicated data Note: Scent marking will be included as a social behaviour since it is meant to mark territory and deter intruders. However, it is also used for mate attraction and can be considered a sexual behaviour. For this review, studies that measure effects on intra-sex scent marking will be included: i.e. the effects of female odours on male scent marking and vice versa will not be included. Scent marking could also be used as an odour treatment, so only effects on intra-sex behaviour will be included.	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	• Article title	

		• Date • Authors • Journal name	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	Control vs treatment designs will be included. Effects of group size on treatment efficacy will be examined. Reported test statistics will be used to calculate study effect sizes using https://www.psychometrica.de/effect_size.html	
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	• Species, breed/strain • Age • Sex	
34.	Intervention characteristics (*e.g.* intervention, timing, duration)	• Treatment route: spray/liquid application vs diffusion • Duration and frequency of treatment • Age at exposure • Exposure environment: home cage with familiar conspecifics vs. testing arena with strangers • Gland of origin	
35.	Outcome measures	• Was there a reported change in behaviour? Which direction? • If stress measures were recorded, was there a reported change? Which direction?	
36.	Other (*e.g.* drop-outs)	• Excluded animals (reason, number) • Cage style: for rodents, ventilated vs static • Bedding material: for rodents, corn cob vs wood chip • Enrichment: was it offered? • Temperature/ humidity: are treatments more/less effective at certain temperatures or humidity? • Behaviour sampling method: all occurrence vs one-zero vs scan • Internal reliability for behaviour recording: was it reported? • Treatment allocation method: was there a randomized allocation reported? • Type of control: did animals receive nothing or a neutral compound (i.e.: water)? • Researcher blinding: was it reported? • Statistical analysis: Was the statistical model described? How was sample size determined?	
	Assessment risk of bias (internal validity) or study quality		
37.	Specify (a) the number of reviewers assessing the risk of bias/study quality in each study and (b) how discrepancies will be resolved	a) 2 reviewers. The criteria will be independently assessed by AJB and an undergraduate assistant by using collectively predefined assessment criteria b) discrepancies will be resolved by discussion or by consulting a third investigator (BNG)	

38.	Define criteria to assess (a) the internal validity of included studies (*e.g.* selection, performance, detection and attrition bias) and/or (b) other study quality measures (*e.g.* reporting quality, power)	X By use of SYRCLE's Risk of Bias tool[4] ☐By use of SYRCLE's Risk of Bias tool, adapted as follows: ☐By use of CAMARADES' study quality checklist, e.g [22] ☐By use of CAMARADES' study quality checklist, adapted as follows: X Other criteria, namely: • use of ROBINS-I for non-randomized studies [15]	
	Collection of outcome data		
39.	For each outcome measure, define the type of data to be extracted (*e.g.* continuous/dichotomous, unit of measurement)	For social behaviour, measures will be divided into sub-categories (aggression, affiliative, investigative) and we will record whether each study reported an increase, decrease, or no effect. For stress measures, we will record whether each study recorded them, which were recorded, and if there was a reported increase, decrease, or no effect. Overall, we will look for efficacy patterns based on species, strain/breed, age, sex, treatment route, testing location, gland origin, and housing parameters listed above. We will also evaluate data quality based on the behaviour sampling method used, if animals were randomly allocated to treatments, the type of control used, whether researchers were blinded to treatment, and the statistical model used.	
40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	We will use published text and graphs. If there is confusion based on what is reported, we will contact authors.	
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	a) 2 reviewers: AJB and an undergraduate assistant b) discrepancies will be resolved by discussion or by consulting a third investigator (BNG)	
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	Descriptive summary of all articles and outcomes.	
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	-	
	If a meta-analysis seems feasible/sensible, specify (for each outcome measure):		
44.	The effect measure to be used (*e.g.* mean difference, standardized mean difference, risk ratio, odds ratio)	-	
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	-	
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	-	
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	-	

48.	Any sensitivity analyses you propose to perform	-	
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)	-	
50.	The method for assessment of publication bias	-	

Final approval by (names, affiliations):		Date:
	Purdue University	6/10/2020
	Purdue University	6/10/2020

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