**Next steps in automated handler-free behavioural recordings: spatially discretized behavioural cycles**

*\*\*\* Dear reader, please note that this is a ResearchEquals ‘Idea’ manuscript, meant to elucidate the ideas behind a behavioural recording device our lab is developing. This represents work in progress, and all feedback is welcome at* *m.m.karnani@vu.nl* *\*\*\**

**Summary**

Open source electronics, code and mechanical devices have enabled a proliferation of automated behavioural recordings which greatly assist neuroscience research. This is particularly important for studying prey species like the mouse, as handler-free measurement of exploitation behaviours will likely capture a different behavioural regime than traditional assays due to the lack of threatening stimuli. Following up on recent discussions of ethological neuroscience1,2, three principles of automated maze development are outlined here, which have helped us to spatially discretize behavioural cycles in a naturalistic way.

**Reasoning**

Motivations for feeding, drinking and social interaction form a scaffold upon which we build our daily lives. In the absence of threat these drives express exploitation behaviours consisting of appetitive and consummatory components3, whereas under increasingly imminent threat, evasion behaviours, escape, hiding, and fighting, must take priority4. This is particularly true for prey species like the mouse. Many sensory stimuli associated with humans can affect the outcome of behavioural experiments on mice by engaging threat responses5. Therefore, automating behavioural experiments to the extent that the experimenter can be removed from sensory detection range can reveal behavioural parameters and neural processing underlying natural exploitative drives6. This idea has fuelled the proliferation of so-called home cage monitoring assays7–9, automated apparatuses for training behavioural tasks10–13, recording parameters of social structure14–16, drug dosing17 and tracking body weight18. Algorithmic pose estimation methods19 also enable handler-free experimentation. These advances have been accelerated by the use of, and sharing as, open source tools20.

To complement new open source devices for standard and advanced learning tasks21–23, and to open up further possibilities for handler-free and ethologically relevant measurements of exploitation behaviours, three key elements merit further development:

1) Voluntary **single entry** of animals into a task or foraging environment is a key element of maze design as it allows the animals to live in the recording setup. This can remove the discontinuity between the home cage and task environments, and the associated affordances such as ad libitum food and water access in the home cage, typically imposed by the experimenter. Single entry, sometimes called animal sorting24, can yield the perfect balance between undisturbed task performance and social interaction. It can also be used to provide social interaction epochs as a reinforcer. The infrequent use of single entry modules10,15,25,26, and the paucity of open source designs, may stem from the potential danger of using automated doors near many animals. We have found that an extremely safe design choice is to use long doors operating upward from the floor in roofless corridors.

2) Mimicking **natural habitats and affordances** spatially and temporally. Mice live in burrows or human houses where they navigate narrow passages to known and unknown affordances27. Mouse burrows tend to include two paths to each chamber28,29, and walled passages are more common than elevated ledges in the natural environments. Therefore, mazes should contain several walled passages linking different areas. Interfacing behavioural measurements with tethered neural recording/manipulation in these environments requires careful design of the passage layout and roofless enclosures, an uncommon design feature, but one that mimics human dwellings.

The temporal availability of affordances should be matched to observed behavioural patterns in the wild as much as possible. It has been suggested that wild mice eat approximately 200 small meals at 20-30 sites throughout the day, mostly during the dark cycle5. However, recent quantifications of pellet retrieval intervals suggest that, per day, laboratory mice eat about 19 meals that last about one minute, consist of about 220 mg of chow and occur most frequently during the dark cycle21. Mice are adapted to arid conditions and can subsist on minimal free water if a food source of at least 10% water content is available, such as dry grain30. Besides their diet, natural water sources are dew, surface water, insects, and sea water. If more than minimal amounts of water are available, mice use it for urine marking31. Laboratory mice with ad libitum access to water drink 3-4 ml of water per day in bouts averaging approximately 0.06 ml occurring most frequently during the dark cycle32.

3) **Discretizing behavioural cycles**. Innate behaviours, like feeding, are cyclic sequences of appetitive and consummatory actions33. Aspects of the cycle may be influenced differently by high need (🡪 prolonged consumption phase) and high availability (🡪 more switching; Figure 1A). To study these effectively, many standardized repetitions are needed. Therefore, the quanta of consumption should be identified and provided in spatially and/or temporally separated epochs. Is the swallowable unit a reasonable quantum, and should species-typical meal-sizes21,34, plant-typical fruit/seed sizes and environment-typical availability5 be accounted for? Devices like the FED3 pellet dispenser21 and open source liquid dispensers35,36 can be used to explore these alternatives, if placed in foraging environments that call into play the naturalistic spatial exploration strategies used by animals in the wild.

These principles have led us to build a simple maze prototype that spatially discretizes behavioural cycles by delivering ethologically-grounded quanta of water or food upon each entry to a water or food area (Figure 1B).

Figure 1, Behavioural cycles, as used in ref 1. A, The stereotypical behavioural cycle. The main influence of need and availability is depicted on different sides of the cycle as need increases the length of consumption bouts37, while changes in availability tend to affect switching to other behaviours38. B, Schematic of how the key areas of development highlighted here can be used to study repeatable discretized behavioural cycles as spatial cycles.

As development of these three elements builds on previous work in the open domain, open source electronics and code, we will make our developments publicly available at no cost. One exciting prospect of developing these devices as an open community is automated non-invasive recordings on freely-participating wild animals, following from previous similar work39,40.

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