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# The touchscreen operant platform for testing learning and memory in rats and mice

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## Summary

An increasingly popular method of assessing cognitive functions in rodents is the automated touchscreen platform, on which a number of different cognitive tests can be run in a manner very similar to touchscreen methods currently used to test human subjects. This methodology is low stress (using appetitive, rather than aversive reinforcement), has high translational potential, and lends itself to a high degree of standardisation and throughput. Applications include the study of cognition in rodent models of psychiatric and neurodegenerative diseases (e.g., Alzheimer's disease, schizophrenia, Huntington's disease, frontotemporal dementia), and characterisation of the role of select brain regions, neurotransmitter systems and genes in rodents. This protocol describes how to perform four touchscreen assays of learning and memory: Visual Discrimination, Object-Location Paired-Associates Learning, Visuomotor Conditional Learning and Autoshaping. It is accompanied by two further protocols using the touchscreen platform to assess executive function, working memory and pattern separation.

## INTRODUCTION

This protocol describes an automated touchscreen platform, in which a remarkable diversity of cognitive functions may be tested in rodents. During more than two decades of research, a number of tasks have been designed and validated for the platform, each allowing the researcher to probe a unique set of functions[1-5]. Together these form a comprehensive "battery" of tasks, several of which may be used in concert by the researcher to elucidate a cognitive profile for a given rodent model, or more selectively to examine specific aspects of the cognitive repertoire in a hypothesis-driven manner.

The touchscreen platform has been used in a number of studies, in a variety of ways. First, putative rodent models of human conditions including Alzheimer's disease[6, 7], schizophrenia[8-10], Huntington's disease[11], frontotemporal dementia (A.E.H., B.A.K., T.J.B. & L.M.S., unpublished results), aging[12], exposure to stress[13] and substance abuse[14] have been studied. Notably, we recently demonstrated the utility of this platform for parallel cognitive testing of humans with schizophrenia and a putative mouse model of the disease (discs, large homolog 2 *(Dlg2)* knock-out) sharing a similar genetic basis[8]. Second, these tasks have been employed to investigate the neural underpinnings of a number of different cognitive functions, targeting the rhinal[15-18], medial and ventromedial prefrontal[13, 19-21], anterior and posterior cingulate[22-26], medial frontal[22, 23], orbitofrontal[13, 27, 28], infralimbic[28] and prelimbic[29] cortices. In the striatum, studies of dorsolateral and dorsomedial areas[13, 20, 21] and the nucleus accumbens[22, 25, 26, 30, 31] have been performed. Roles for a number of other brain regions, including the amygdala[25, 32], distinct thalamic nuclei[29], the subthalamic nucleus[33], the fornix[17, 34], subiculum[32], hippocampus[27, 35-41], pedunculopontine tegmental nucleus[42], medial septal/vertical limb of diagonal band (cholinergic neurons)[43] and nucleus basalis magnocellularis (cholinergic neurons)[44] have also been identified in a number of tasks. Third, the efficacy of systemic pharmacological agents has been studied, using compounds active on the cholinergic[7, 45-48], dopaminergic[14, 31, 48-50], glutamatergic[9, 48] and serotonergic[50-53] systems. Fourth, the function of specific genes[8, 51, 54, 55], receptors[56], receptor subunits[57-59] and structural plasticity processes such as adult hippocampal neurogenesis[12, 60] have been assessed.

### Advantages and disadvantages of the touchscreen platform

The advantages of the touchscreen platform have been discussed in detail elsewhere (see [1-3]). Briefly, this platform offers the potential for a very high degree of standardisation, minimal experimenter involvement and high translational potential (e.g., similarity to human CANTAB (Cambridge Neuropsychological Test Automated Battery) tests). It includes

assays of various neuropsychological constructs, including attention and cognitive flexibility, and utilises appetitive rather than aversive motivation. One obvious advantage of using computer-generated visual stimuli is that the perceptual features (size, shape, contrast, luminance, etc.) and similarity of the stimuli can be easily manipulated[3, 61]. Furthermore, in object-based tasks in which the objects are displayed in different locations on the touchscreen, there is no potential for use of odor cues, unlike some (dry) maze tasks, which can modify results. The platform also lends itself to applications that allow for the measurement of brain functions, *in vivo* as animals perform a task, for example via single-unit neuronal recordings[62]. There is potential for the incorporation of other powerful methodologies (e.g., optogenetics) to the touchscreen platform. Whilst we focus on rodents in this article, touchscreens have been utilised with pigeons and non-human primates, as well as with mice and rats, e.g.,[2, 63-68].

It is worth noting that although automated methods such as the touchscreen platform reduce experimenter effort, the tasks can take many more sessions to run than equivalent tests using, e.g., odors. However, because large numbers of animals (>20) can be run in parallel, experiments can often be completed in the same number of days, or fewer, as they can using "hand-testing" methods in which an experimenter tests one animal at a time. Furthermore, while the hand tester is working on their one experiment all day, the experimenter with a number of automated units can work on several. Of course, to achieve this high throughput, one needs the apparatus, which means a larger initial financial outlay than required for most "hand-testing" methods. Again, however, if one considers all factors, for example salaries and person-hours spent on experiments, and the fact that such apparatus can be used for many years before needing to be replaced, in the long run automation may actually be less expensive than hand-testing alternatives.

Another potential limitation is that the use of visual stimuli precludes the use of certain subjects, for example mice with genetic alterations that cause rapid retinal degeneration. (Albino rats, however, appear to have sufficient acuity to perform as well in the touchscreen as pigmented rats[3].) In addition, as with most appetitive, operant paradigms, the use of food reward may introduce possible problems; for example, an experimental treatment may affect appetite or interact with the physiological effects of food restriction. These limitations should be kept in mind, although we do believe that all things considered, the advantages conferred by avoiding aversive stimuli far outweigh the disadvantages of using appetitive stimuli. Touchscreen tasks require intact motoric function such that subjects are able to traverse the testing chamber, respond to the screen, and collect and consume food reward. Again, however, these demands are much lower than many currently used behavioral paradigms. Importantly, the impact of most of these potential changes can be assessed by taking a "battery" approach, by running appropriate control experiments and/or inspecting relevant dependent variables such as trial omissions and/or reaction times to respond or to collect the reward. If one takes a battery approach, testing the effect of a given experimental manipulation upon several tasks, then the tasks can act as mutual controls by virtue of the fact that they involve the same types of apparatus, stimuli, responses and reinforcement[1]; comparisons can be made confidently between tasks in the battery because such variations are minimal. For example, if an animal performs poorly in Object-Location Paired-Associates Learning (which theoretically requires cognitive functions including visual discrimination and learning of object-location associations; discussed further below), but well in Visual Discrimination (which requires visual discrimination learning; discussed further below), it would be reasonable to conclude that the former impairment is not due to a general problem in perceptually discriminating images. Similarly, we have found that muscarinic M2 receptor knock-out mice are impaired on Object-Location Paired-Associates Learning, but actually demonstrate improved attention in the 5-Choice Serial Reaction Time

(5-CSRT) task (Romberg, C., et al., unpublished results), making it very unlikely that the former impairment is due to an attentional deficit.

Finally, for researchers for whom the "ethological validity" of a method is important, rodents using touchscreens may not be the method of choice. However, we would note that the behavior in the touchscreen is built on the natural tendency of rodents to approach and explore novelty in their environment; the exploration is detected by the touchscreen, and the animal learns, again quite naturally, the consequences of exploring certain stimuli. In this sense the method is no less "ethologically valid" than rodents swimming in an artificial pool in a laboratory setting, or other commonly used laboratory methods. In any case, we see the touchscreen method as complementing, rather than replacing, other methods such as foraging paradigms etc.

## Assessing learning and memory

This protocol describes four tasks which may be used to assess aspects of learning and memory. The first three of these rely primarily on appetitively-motivated instrumental learning, and are preceded by "*pretraining*" in which subjects must learn to make instrumental responses in the touchscreen apparatus. Visual Discrimination is a relatively simple task, in which subjects must learn to consistently respond to one of two visual stimuli. In Object-Location Paired-Associates Learning, the correct stimulus is identified by the conjunction of a visual stimulus and its location on the touchscreen. In Visuomotor Conditional Learning (VMCL), the correct response (left or right) depends on which conditional visual stimulus is presented. Autoshaping is unique in the battery, primarily testing Pavlovian stimulus-reinforcer learning. Two accompanying protocols discuss additional tasks which may be used to assess working memory and pattern separation[4] (Trial-Unique Non-matching to Location (TUNL) and Location Discrimination (LD)) and executive function[5] (Reversal, Extinction and 5-Choice Serial Reaction Time (5-CSRT) task). Other tasks are constantly in development, which will expand the range of the battery yet further.

**A) Visual Discrimination—**Learning to discriminate between environmental stimuli is essential to successfully shape decisions and adaptively guide behavior. Understanding the neural mechanisms supporting discrimination learning is of significant interest to cognitive neuroscience and could have implications for delineating the pathophysiology of cognitive impairments in neuropsychiatric disorders from schizophrenia to Parkinson's disease. Basic preclinical research in animals is key to this work, and various methods for testing discrimination learning have been developed, including touchscreen-based systems in non-human primates[69]. In addition to the basic pair-wise discrimination procedure, certain variations have also been developed, including multidimensional[70] (to test attentional set-shifting), concurrent (e.g., [23]) and conditional (see VMCL) discriminations, and transverse patterning[34] (to test configural learning).

Initial studies using a touchscreen discrimination procedure were published almost twenty years ago, using a configuration of a monitor, off-the-shelf operant hardware and customized software[2] (see also[71]). Briefly, the procedure entails simultaneous presentation of two stimuli, and the measurement, over multi-trial sessions, of the animal's ability to reliably touch the stimulus designated the CS+ (rewarded) in favor of the other stimulus (CS −, non-rewarded). Discrimination learning requires at least two processes: learning to perceptually discriminate the stimuli, and learning which of the two stimuli is associated with reward. It also provides the basis for testing reversal learning (see [5]), in which the stimulus-reward contingencies acquired during discrimination are reversed.

The task has been employed to investigate a variety of questions concerning the neural basis and pharmacological modulation of visual discrimination learning. These include testing the effects of drug treatments, ranging from psychotomimetics and putative cognitive enhancers[9, 13, 18, 47], gene mutations, particularly of glutamate signalling molecules[8, 10, 51, 55, 57-59], discrete brain lesions[13, 23, 28, 72-74], and environmental manipulations such as exposure to stress[13].

**B) Object-Location Paired-Associates Learning—**The formation of an association between two individually neutral stimuli, named paired-associate learning (PAL), has been extensively studied in humans using a variety of modalities (verbal, visual, locations). Whilst PAL has traditionally been assessed using pairings of words tested by cued recall, the human CANTAB PAL task[75] does not rely on verbal stimuli and thus provides a version of PAL which is more amenable to modelling in animals. The computerised PAL task requires the subject to form an association between a visual stimulus and its location on a screen, demonstrated under cued-recall conditions. Over more than two decades, CANTAB PAL has been validated as sensitive to detecting deficits in a range of conditions such as schizophrenia[76-78], Huntington's disease[79], Parkinson's disease[75], major depressive disorder[80], unipolar and bipolar mood disorders[81] and Alzheimer's disease[75, 79, 82-86].

Given the profile of neuropsychiatric disorders to which object-location learning is sensitive, it is not surprising to find that encoding and retrieval of object-location associations has been linked to hippocampal and prefrontal cortical function[87-89]. Importantly, the same areas have been implicated in the rodent touchscreen Object-Location Paired-Associates Learning task developed by John Talpos[41, 90], in which the animal is required to learn three individual object-location associations. Each visual stimulus ("object") is correct in a unique location, which stays stable throughout training. On each trial, two different objects are presented, one in its correct location, the other in an incorrect location. The third location remains blank. The rodent task differs from that of CANTAB PAL in that the stimuli are not trial-unique, and the task does not feature a delay. Importantly however, the requirement to use both object and location information to solve the task is maintained. Indeed, assessment of paired-associates learning in patients with *DLG2* mutations using CANTAB PAL produced a similar phenotypic profile to that of *Dlg2* knock-out mice using the rodent Object-Location Paired-Associates Learning task[8], indicating the translational potential of the paradigm. We note that in this task, the animal can approach locations on the screen from many different angles, which is in contrast to the behavior that we see in, e.g., the VMCL task (see below).

Pharmacological manipulation of the rodent Object-Location Paired-Associates Learning task indicates that both facilitation and disruption of performance is possible. Antagonism of NMDA or AMPA receptors in the hippocampus impairs performance in rats, but leaves accuracy unaltered for a similar control task which may be solved by visuomotor conditional learning as opposed to the formation of object-location associations[41]. Systemic pharmacological manipulations in mice have further implicated cholinergic muscarinic receptors in performance of the task, with a facilitation observed in wildtype animals using donepezil[45]. Muscarinic M2, but not M1, receptor knock-out impairs acquisition of the task[56] (Romberg, C., et al., unpublished results). Task performance is sensitive to amphetamine but not PCP, ketamine or LSD[91]. Thus, the task offers an automated and sensitive measure of rodent object-location paired-associates learning and performance, which has translational potential.

**C) Visuomotor Conditional Learning—**In VMCL, animals learn a conditional rule of the type "If visual stimulus A is presented, make motor response X; if visual stimulus B is presented, make motor response Y". There has been significant interest in such "visuomotor

mapping" in the primate[92]. Generally it appears that across monkeys and rodents, hippocampal damage does not consistently produce impairments in such tasks, although the hippocampal system can become involved when mappings are acquired rapidly or involve object-location rather than visuomotor associations (see[93, 94]). Rodent VMCL in operant chambers requires discrete left-right responses and thus likely involves visuomotor associations, which likely require stimulus-response habit learning, and as would therefore be expected, the task is more sensitive to damage in the striatum than the hippocampus[95, 96]. The VMCL task in the touchscreen is indeed designed to maximize stimulus-response learning and minimize other cognitive demands. Thus, the discrimination is chosen to be an easy one (in practice probably solved via light-dark discrimination), to reduce perceptual demands. Furthermore, a "limited hold" (time limit) for responding promotes the same rapid head-turn-and-nose poke motor response on each trial, encouraging a visuomotor strategy, and limiting the extent to which subjects can move away from the screen and reapproach the choice stimuli from different angles, which might promote alternative learning strategies. Touchscreen VMCL does not require medial prefrontal cortex, anterior cingulate cortex, hippocampus, perirhinal cortex, anterior thalamus or mediodorsal thalamus, but does depend on posterior cingulate cortex (late in learning only)[23, 74, 97], thus conferring the specificity needed to dissociate function as part of a touchscreen test battery (for an example see[23]). Like pair-wise visual discrimination learning, the task can also be "reversed" to engage a different set of brain regions[22, 43, 74]. The VMCL task may be particularly relevant to Huntington's and Parkinson's disease, in which cognitive impairments include deficits in habit learning[98, 99].

**D) Autoshaping—**The Autoshaping task assesses Pavlovian approach learning. It capitalises on the process of "autoshaping" which was first observed in experiments in which pigeons came to reliably peck at an illuminated key (conditioned stimulus; CS) presented immediately before delivery of grain at a separate location[100], and has been reported in many species[8, 101-106]. It is considered to rely on Pavlovian, as opposed to instrumental, associations[22]. While a behavioral chamber equipped with levers can be used to assess rodent autoshaping[107], this protocol details the use of a touchscreen system as originally described by Bussey and colleagues[22].

Autoshaping is a discriminative conditioning procedure, in which a stimulus is presented on either the left or right side of the touchscreen, with one side defined as CS+ (rewarded CS) and the other as CS− (non-rewarded CS). Reward is delivered upon termination of the CS+, but not CS−. With repeated presentations, rodents increase CS+ approaches and decrease CS − approaches, indicating that the predictive relationship between CS+ presentation and reward delivery has been learned[22]. To demonstrate the Pavlovian nature of the association, a reward omission procedure[22, 108] can be implemented in which CS+ approaches cause reward to be withheld. Under this altered contingency, animals continue to respond to the CS+, which is consistent with a Pavlovian CS-UR association[22, 108].

This task requires minimal pretraining and animals quickly develop the necessary stimulus discrimination, making it relatively rapid to complete. Therefore, it has been used extensively to characterise the neurobiological mechanisms underlying Pavlovian learning in conditions in which the effects of instrumental learning mechanisms on performance should be minimised[26]. In particular, studies of rodent autoshaping after disruption of defined brain regions have identified critical roles for the nucleus accumbens core[25, 26, 109], anterior cingulate cortex[22, 24, 25] and the projections between them[22, 25, 26, 110]. A number of other structures, including the orbitofrontal cortex[28], central nucleus of the amygdala[32], pedunculopontine tegmental nucleus[42] and subthalamic nucleus[33] are also required. Lesions of the hippocampus appear to enhance autoshaping acquisition[36]. The anatomical specificity of this task is striking, as disruption of other closely related brain regions, such as the

basolateral nucleus of the amygdala[32], nucleus accumbens shell[26], dorsal striatum[20, 21], posterior cingulate cortex[22], medial prefrontal cortex[20-22] and infralimbic cortex[28] have no effect. This task is also sensitive to systemic administration of a number of pharmacological agents, including typical and atypical antipsychotics[111] and apomorphine[31]. Central administration of a variety of neurotransmitter receptor antagonists has indicated that functional glutamatergic and dopaminergic accumbens signalling is required[30, 107]. These features make the task valuable in furthering understanding of stimulus-reinforcer learning generally, and particularly if the reinforcer is maladaptive, as in drug addiction[25, 107]. It has also been suggested that aspects of the task can model impulsive and perseverative responding[33, 53]. The strong dependence of the task on dopaminergic and glutamatergic signalling may also be of value in studies of conditions in which these are disrupted, such as schizophrenia[112, 113], with potential for relatively rapid screening of novel rodent models or therapeutics. Furthermore, one could conceivably monitor magazine entry during stimulus presentation to measure goal-tracking in addition to sign-tracking behavior (e.g.,[114]). We are currently exploring this possibility, which may prove particularly useful for models of neuropsychiatric disease. For example, Danna and Elmer found that the atypical antipsychotic olanzapine and typical antipsychotic haloperidol disrupted conditioned approach to a reward-predictive cue (sign-tracking) but neither drug disrupted conditioned approach to the reward (goal-tracking)[111]. Furthermore in the context of drug addiction, it has also been shown that differences in sign-tracking and goal-tracking can reflect underlying differences in the dopamine system[114] and is linked to responsiveness to drugs of abuse[115].

## EXPERIMENTAL DESIGN

### General considerations

Task-specific experimental details are described below in sections dedicated to each task. Unless stated otherwise, the tasks are described here in the way that we presently conduct (or intend to conduct) them. In this first section, some general principles, advice and alternatives are discussed.

**Apparatus type—**We use two types of touchscreen apparatus: in-house assembled apparatus, and apparatus commercially available from Campden Instruments Ltd. Both are described in MATERIALS. The majority of tasks presented here have been performed in both.

**Houselight—**Our current standard procedure is to have the houselight off during stimulus presentation and inter-trial intervals (and on for "*time out*" periods), but the majority of tasks have also been performed with the houselight on, and we do not have conclusive evidence that these variations affect task performance.

**Reward—**Two types of reward are typically used – liquid or solid (see MATERIALS). Pellets seem to work well for rats. We use either liquid or solid for mice; liquid rewards may be a better choice in some cases, e.g., when using manipulations that result in motoric changes that could affect chewing, cause dry mouth, or reduce motivation.

**Inter-trial intervals (ITIs)—**The "*ITIs*" in the tasks presented in this paper are 20 s (except for Autoshaping). Although shorter ITIs are frequently used, particularly with mice (e.g., 15 s[13, 51], 5 s[19, 49, 57, 70]), longer ITIs may facilitate learning[3].

**"Free" initial reward delivery—**In the majority of touchscreen tasks (excluding Stages 1-3 of pretraining, Autoshaping and Extinction), a "free" reward is delivered (e.g., one

reward pellet or 20 μl milkshake) at the start of each session to prime responding and encourage initiation of the first trial. This may be delivered manually prior to the start of the session, or automatically at the start of the session by the software program.

**"*Correction trials*" (CTs)—**When the subject makes an incorrect response, the next trial initiated will be a CT (in the majority of tasks; see task-specific EXPERIMENTAL DESIGN), in which the same stimulus/stimuli are re-presented in the same location(s). CTs do not count towards the session trial limit, or the main accuracy score (see data analysis below). There is usually no limit on the number of CTs that can be given consecutively, but once the subject responds correctly, the correction procedure ends. The purpose of CTs is to counteract side and stimulus biases, and to ensure that subjects receive a consistent number of rewards per session despite their performance on non-correction trials.

**Data analysis—**There are several performance measures common to the majority of touchscreen tasks. The measures recorded for each animal in each session of these tasks include: number of responses to blank/correct/incorrect stimuli (for correction and non-correction trials separately), total number of trials and CTs completed, correct/incorrect response latency and reward retrieval latency. From these, the following measures may be calculated for each phase of an experiment: *Percentage accuracy* (100*(correct responses)/(correct + incorrect responses)), which is often plotted as a function of session, i.e. an acquisition curve. Note that this measure does not include CTs.

*The number of sessions/trials/errors (incorrect responses in non-correction trials) to attain a specified performance criterion.*

*Average latency to make a correct/incorrect response*, following presentation of stimuli (also termed "reaction time"). Note that data from CTs are not usually included in this measure.

*Average latency to collect reward* after a correct response is made (also termed "magazine latency"). Latencies to respond and collect reward (usually in non-correction trials only) can reveal perturbations in motivation, motoric function, speed/error trade off, etc.[116].

Where bias towards a specific location/stimulus may affect responses (e.g., Visual Discrimination, VMCL), *percentage of bias* can be calculated, e.g., for the first session. This is the number of trials in which the subject responds to a particular location/stimulus, expressed as a percentage of all trials. In cases in which a treatment affects innate stimulus bias, assessing rate of task acquisition will be problematic as the treatment and control groups will not be at similar performance levels (i.e., chance) at the outset of the experiment.

A *perseveration score* (also termed "perseveration index") may be calculated to assess the extent to which subjects perseverate in responding to the incorrect location/stimulus during CTs after an incorrect response, corrected for the number of initial incorrect responses (on first presentation trials). This may be expressed as the average number of CTs per incorrect response

*Screen touches during ITI/time out* may be calculated, and might provide an additional measure of perseveration or motoric activity.

If performance is expected to vary within-session, for example after drug administration, it may be useful to analyze the above mentioned measures in bins of, e.g., 10 trials

**Experimental manipulations—**In all of the tasks described here, the specific research question and experimental manipulation determines the behavioral procedure. For clarity, we will describe 4 possible treatment scenarios. In Case 1, the subject receives treatment before onset of the experiment (e.g., constitutive transgenic or knock-out models, developmental manipulations). In Case 2, the subject receives treatment before task acquisition, but after pretraining (e.g., subchronic drug treatment, neurotoxic lesions). In Case 3, the subject receives treatment after acquisition to assess effects on asymptotic performance level, or on post-acquisition behavioral challenges, using a between-subject design (e.g., neurotoxic lesions, subchronic drug treatment). In Case 4, the subject receives a transient manipulation at asymptotic performance level, or during post-acquisition behavioral challenges, that can be performed within-subject (e.g., systemic pharmacological or infusion procedures). We will refer to these cases as appropriate in our protocols.

When post-acquisition manipulations are of interest (including Cases 3 and 4, and post-acquisition behavioral challenges), there are several options for the point at which animals should be advanced from acquisition training. First, a group of animals may all be tested for a prespecified number of acquisition sessions, and then all advance to the post-acquisition manipulation regardless of performance level. An advantage is that all animals in the group will be synchronised i.e. the manipulation will begin for all animals on the same day, which minimises variability due to extraneous factors, is ideal for pharmacological studies (because injections (whether vehicle or drug for a given animal) may be conducted on the same day(s) for all animals) and enables decisions (e.g., concerning number of days to run a manipulation for) to be made *ad hoc* based on the group's mean performance level. This is also particularly important when subjects must be the same age at the start of each testing phase, for example, when testing a progressive disease model. However, there will be some variation in the performance levels of the animals at the end of training, and some may not have acquired the initial task to a sufficient baseline level from which to assess alterations in performance due to a manipulation.

Second, a group of animals may be trained until all animals in the group have reached a performance criterion. However, whilst this means that the group will be synchronised and have the same number of training days prior to the post-acquisition manipulation (allowing for an acquisition curve to be plotted), some animals will be over-trained.

Third, each animal in the group may be trained until it reaches criterion, then individually advanced to the manipulation of interest. Whilst this avoids over-training and variations in performance level, the group is not synchronised.

We suggest a fourth option: each animal in the group is trained daily (at least 5 days per week) until it reaches criterion, upon which it is "rested" without daily training (although food restriction continues). Subjects on rest are usually given one or two "reminder" training sessions per week unless it is anticipated that all subjects will reach criterion within a few days of each other. If an animal's performance falls below criterion in a "reminder" session, that animal is trained daily until criterion is reattained. When all animals have reached criterion (at least) once, they are rebaselined as a group i.e. all animals are trained daily. Post-acquisition manipulations may begin when performance of all subjects has been stable at criterion for at least two days. Whilst subjects receive a different number of training days, precluding plotting of a complete acquisition curve, the animals are synchronised, with minimal variation in their performance levels, and over-training is minimised.

**Flexible battery approach—**The tasks presented in this set of articles[4, 5] form part of a "*flexible battery*"[1], meaning that the tasks and task order employed can be tailored by the researcher to address specific hypotheses and research requirements. Although not suitable

for all types of manipulation (e.g., progressive disease models, drug studies), we suggest using a “battery approach” to elucidate a cognitive profile, where appropriate. This approach is particularly suitable when there are no specific hypotheses regarding the domains of cognition that will be affected by a manipulation. Here, a single group of animals is tested on multiple tasks from the battery, as well as probes if appropriate (see post-training manipulations in task-specific PROCEDUREs). In comparison to the other extreme of testing a naive cohort on each task, this battery approach requires fewer animals and is more efficient (full pretraining is only required before the first task), although further research is necessary to explore order effects and the potential for negative or positive transfer between different task combinations. We have settled upon two mini-batteries of tasks for mice, which comprise the six most commonly used tasks available. (Autoshaping is usually tested in a dedicated cohort – see below.) One cohort is tested upon Visual Discrimination, Reversal, Object-Location Paired-Associates Learning and Extinction, and a second is tested on 5-CSRT and LD[8]. Using these sequences, we have not observed significant transfer effects in control or experimental groups of mice. However, order effects may only be conclusively ruled out by retesting naive animals on the task in question. Order effects are of course an important consideration for all cognitive and behavioral testing of rodents, where the same animals are tested on more than one task.

## Pretraining

All tasks in the touchscreen battery are motivated by food reward, and the majority require instrumental responses to the touchscreen. Therefore, to provide sufficient motivation, animals are subject to mild food restriction prior to task training. Pretraining normally consists of five stages followed by training specific to the task. As described previously (see, for example,[6-8, 12, 51, 63]), these gradually shape the screen-touching behavior required by all of the instrumental touchscreen tasks (an exception is the Pavlovian Autoshaping task; see below). The number and size of response windows and the size and type of visual stimuli used during pretraining depend on the task that the subject is to be trained on subsequently. If that task uses plain white square stimuli, including VMCL, the pretraining stimulus is usually a plain white square. For other tasks which use discriminative stimuli only (including Object-Location Paired-Associates Learning, Visual Discrimination), pretraining stimuli are from a library of 40 varied black and white shapes, none of which substantially resemble the stimuli used in these tasks. The rationale for this procedure is that generalization between the training and task-specific stimuli should be minimized. Number and size of response windows and stimuli for tasks in the paper can be found in Table 1. We note that rats are typically given the opportunity to complete more trials per session than mice, e.g., 100 as opposed to 30 during pretraining. Rats readily complete a greater number of trials per session than mice, perhaps because the mouse:rat body mass ratio is smaller than the mouse:rat reward pellet size ratio (14mg:45mg).

Following the introduction of mild food restriction, animals are habituated to the chambers and food rewards for at least 2 daily sessions (see PROCEDURE; Stage 1). In Stage 2 (see Fig. 1), the relationship between offset of a visual stimulus on the screen and delivery of reward is introduced. A stimulus is presented in one of the response windows (with the same location not used more than 3 times consecutively). If not touched, offset occurs after 30 s and a reward is delivered, along with illumination of the magazine and a tone (e.g., 1s, 3 kHz; conditioned reinforcer). Touches to stimuli on the screen are encouraged with immediate offset, a triple reward delivery, tone and magazine illumination. When the animal enters the magazine to retrieve the reward, the magazine light is turned off and an ITI begins, after which the next trial is automatically initiated.

Stage 3 (see Fig. 1) is similar to Stage 2, but stimulus offset is dependent upon the subject touching it. A stimulus is presented in one of the response windows, and remains there until

touched, upon which the stimulus disappears and a reward is delivered accompanied by a tone and magazine illumination. When the animal enters the magazine to retrieve the reward, the magazine light is turned off and an ITI begins, after which the next trial begins automatically.

Stage 4 (see Fig. 1) is similar to Stage 3, but subjects are required to trigger stimulus presentation, referred to as trial *initiation*. The session begins with a "free" reward delivery and magazine illumination, indicating that a trial may be initiated. When the animal nose pokes into the magazine, the magazine light is extinguished and a click sounds (0.2 s), and when the animal withdraws from the magazine, stimuli are presented on the screen. Initiation is also required after each ITI.

Stage 5 (see Fig. 1) is similar to Stage 4, but subjects are discouraged from touching blank response windows during stimulus presentation, with stimulus removal and a 5 s time out period in which the houselight is inverted. After the time out, an ITI begins, after which the next trial can be initiated. However, in pretraining preceding the instrumental tasks in this paper, a CT is given instead of a new trial (see General considerations above). This stage also serves to introduce the subject to the cue signalling incorrect responses (the time out). By the end of pretraining, subjects should be completing a sufficient number of trials per session (as specified in PROCEDURE), to promote completion of sessions in the subsequent task.

Analysis of pretraining performance is minimal. The number of sessions required to complete each phase of pretraining, or the overall number of sessions required to complete pretraining, may be analyzed[6-9, 12, 51, 52, 55, 57, 59, 63]. Additionally, if using apparatus that permits assessment of activity in the chambers, measurements pertaining to this (e.g., number of beam breaks per half hour) may be analysed too.

### A) Visual Discrimination

This protocol is based on recent mouse and rat publications (e.g.,[6, 8, 13, 47, 58]) with minimal changes. A Visual Discrimination session (see Fig. 2) begins with a "free" reward delivery and magazine light illumination, indicating that a trial may be initiated (as in pretraining). After initiation, two stimuli (CS+ and CS−) appear in the two response windows. The locations of the CS+ and CS− are pseudorandom, with the stimuli not displayed in the same locations for more than 3 consecutive trials (excluding CTs). The reward contingencies may be counterbalanced, such that for some animals a given stimulus will be CS+ and the other CS−, whereas for other animals the reverse will be true. If the animal touches the CS+ (correct), the stimuli are removed and a reward is delivered along with illumination of the magazine light and a tone (1 s, 3 kHz). When the animal enters the magazine to retrieve the reward, the magazine light is turned off and an ITI begins, after which the magazine is again illuminated to indicate that a new trial may be initiated. If the animal touches the CS− (incorrect), the two stimuli are removed and the houselight is inverted for a 5 s time out period, after which an ITI begins, and then the next trial may be initiated. However, instead of a new trial (as would be presented after a correct response), a CT is given (see General considerations, above).

Various training stimuli may be used (see Fig. 3). For rats, the "Spider-Plane" pair (Fig. 3a) is typically used[3, 47]. For mice, the "Marble-Fan" (Fig. 3b) pair (used in the majority of previous publications[6, 8, 11, 13, 55, 59, 63]) is typically used in our purpose-built apparatus. Both rats and mice are also able to discriminate complex photographic stimuli[3, 8, 56, 59] (e.g., Fig. 3d). We have also recently developed "Lines-Grid" (see Fig. 3c and Supplementary Video 1) stimuli in Campden apparatus, which optimise rate of acquisition but minimise stimulus bias in that apparatus. Depending on the hypotheses under investigation, morphed

stimuli (e.g., Fig. 3e) with over-lapping features[47, 48, 56] can be employed to increase the difficulty of the discrimination, usually as post-training behavioral challenges once subjects have acquired the initial discrimination. These may reduce possible ceiling effects and thereby increase the potential for detecting experimentally-induced improvements[47]. Tests under various difficulty levels also allow examination of interaction between task difficulty and the experimental manipulation[48]. We note that there are several examples in the literature of alternative visual discrimination stimuli, apparatus and experimental designs, e.g.,[35, 46, 48, 61, 65, 71, 117, 118]. Another option is to train subjects on several pairs (e.g., 3 pairs[63], 4 pairs[16] or 8 pairs[2, 16, 23]) of stimuli concurrently, combining trials of each pair within each session; the basic procedure for concurrent discrimination learning is identical to that provided below. In some cases pair-wise Visual Discrimination may serve as the first stage in a more complex task, such as transverse patterning[34]. Another possible post-training manipulation is to retest animals after a delay (e.g., 5-7 days) with the same set of stimuli with which they were trained, to test retention. Retention tests can be used to assess the effects of pharmacological or other manipulations on previously acquired visual discriminations[14], or to test hypotheses about the nature of acquisition learning[6]. Note that it is possible to test the same group of animals on more than one discrimination, e.g., to test mice with “Marble-Fan” stimuli followed by photographic stimuli, although transfer effects are possible (see discussion of the “flexible battery” approach in General considerations, above).

Typically, Visual Discrimination acquisition performance is assessed in terms of percentage accuracy in the form of an acquisition curve, and/or in terms of the number of sessions, trials and errors (incorrect responses on non-correction trials) required to reach criterion. Additionally, latencies, percentage of bias and perseveration score may be analyzed. We refer the reader to General Considerations and PROCEDURE for further details.

### B) Object-Location Paired-Associates Learning

An Object-Location Paired-Associates Learning session (see Fig. 4) begins with a “free” reward delivery and illumination of the magazine light, indicating that a trial may be initiated (as in pretraining). After initiation, two stimuli are presented, composing one of six trial types (see Fig. 5a). A response can be made to the S+ (object in correct location i.e. a correct response) or the S− (object in incorrect location, i.e. an incorrect response). Following a correct response, the stimuli are removed from the screen and a reward is delivered in conjunction with a tone (1 s, 3 kHz) and magazine illumination. When the animal enters the magazine to retrieve the reward, the magazine light is turned off and an ITI begins, after which the magazine is again illuminated to indicate that a new trial may be initiated. Following an incorrect response, the stimuli are removed from the screen and the houselight is inverted for a 5 s time out period, after which the ITI begins. Following the ITI, the magazine is illuminated for trial initiation, but the next trial will be a CT (see General considerations, above). Excluding CTs, there are an equal number of presentations of each trial type in each session, in a pseudorandom sequence (maximum 3 consecutive presentations).

This protocol for rats and mice is based on that first described by John Talpos and colleagues[41], using the “Flower-Plane-Spider” stimulus combination described in published work (see Fig. 5a). However, we note that recent rat task development has led us to use line patterns as “objects” instead (see Fig. 5b), based on preliminary data indicating reduced variability when using patterns compared to images. Additionally, whilst we present the task here with no consequences for touches to the blank location when stimuli are presented on the screen (as in previous publications), we are currently using a method in which we follow blank touches by stimulus offset and a CT (see Fig. 4).

In order to test whether animals form specific object-location associations during the task – as opposed to acquiring a set of trial type-specific "conditional" responses – one can run a probe test in which trials consist of the presentation of two copies of the same object, one in that object's correct location, the other in one of that object's two incorrect locations (e.g., S + = object 1 in location 1, S− = object 1 in location 2, referred to as sPAL (samePAL) in[41]). Whilst Talpos and colleagues assessed the difference between the standard Object-Location Paired-Associates Learning task training and the same-object probe using a between-subject design[41], the common approach developed since involves running two sessions of the same-object probe after stable performance on the standard task has been established. The degree to which an animal's performance drops during this probe test is interpreted as reflecting the extent to which the animal was solving the original task according to alternative, non-configural strategies.

Typically, Object-Location Paired-Associates Learning acquisition performance is assessed in terms of percentage accuracy in the form of an acquisition curve, and/or in terms of the number of sessions or trials required to reach criterion (see ANTICIPATED RESULTS). Additionally, errors, latencies, percentage of bias and perseveration score may be analyzed. In addition to these performance measures, preliminary evidence from our lab indicates that all trial types are not always acquired at an equal rate, particularly "Flower-Plane-Spider" stimuli when acquired by rats (see Fig. 5a). Therefore, separate trial-type performance analysis may be performed, since performance differences may be more pronounced depending on the trial type. We refer the reader to General Considerations and PROCEDURE for further details.

### C) Visuomotor Conditional Learning

The protocol described here is the most recent for rats; the task is still in development for the mouse. Building upon previous publications[2, 23, 74, 97], the present protocol includes an additional phase of VMCL-specific pretraining following standard pretraining, and immediately prior to VMCL training. This phase addresses several potential concerns. First, it counteracts any initial side bias that subjects may have, by requiring responses to both flanking locations. Second, it accustoms the subject to making two responses for a reward, which is in contrast to the single response required for reward during pretraining. Third, it provides an opportunity to introduce a *Limited Hold* (LH) period.

Each VMCL-specific pretraining session begins with a "free" reward delivery and illumination of the magazine light, indicating that a trial may be initiated (as in pretraining). After initiation, a plain white square is presented in the central location, which remains on the screen until touched (touches to the two blank locations are ignored). When the central stimulus is touched by the subject, it disappears and is replaced by another stimulus (also a plain white square) in one of the two flanking locations (left and right; 1 and 3). Excluding CTs, the same location is not used more than 3 times consecutively, and each location is used in 5 out of every 10 trials. This second stimulus remains on the screen for the LH period (usually 2 s), or until a response is made. Touches to the central location are ignored. Following a correct response (stimulus touched within the LH period), the stimulus disappears, a reward and tone (1 s, 3 kHz) are delivered, and the magazine is illuminated. When the animal enters the magazine to retrieve the reward, the magazine light is turned off and an ITI begins, after which the magazine is again illuminated to indicate that a new trial may be initiated. Following an incorrect response (blank peripheral location touched within the LH period), the stimulus disappears, the houselight is inverted for a 5 s time out period, and then the ITI begins. After the ITI, the magazine is illuminated and a CT may be initiated (see General considerations, above). If the subject fails to respond during the LH period, consequences are the same as for an incorrect response. The purpose of the LH is to ensure

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that the subject responds to the flanking stimulus whilst still at the screen after making the initial response to the central stimulus, e.g., the rat makes a head turn whilst rearing.

VMCL task trials progress in a similar manner (see Fig. 6). However, instead of a plain white square stimulus in the central location, one of two discriminative stimuli is presented (see Fig. 7). Excluding CTs, the same stimulus is not used more than 3 times consecutively, and each stimulus is used in 5 out of every 10 trials. When the subject touches the discriminative stimulus, it remains on the screen and two "choice" stimuli are also presented: a plain white square in each of the two flanking locations (left and right). These remain on the screen until one is touched, or until the LH (2 s) is exceeded. Touches to the discriminative stimulus are ignored. The nature of the stimuli is counterbalanced but, for example, if Stimulus A is presented, the left stimulus is correct, and the other incorrect, whereas if Stimulus B is presented, the right stimulus is correct. Again, three response types are possible; the definitions and consequences of these are as in VMCL-specific pretraining, except that an incorrect response is now defined as a response to the incorrect stimulus (rather than to the blank peripheral location).

Depending upon the aims and hypotheses of the researcher, the reward contingency may be reversed after acquisition, to test reversal learning (and thereby assess cognitive flexibility)[74].

Typically, VMCL performance is assessed in terms of percentage accuracy in the form of an acquisition curve (if all subjects complete a certain minimum number of sessions, e.g.,5, 10)[74, 97], and/or in terms of the number of sessions, trials and errors required to reach criterion[23, 74, 97]. Additionally, average correct and incorrect response latency, average magazine latency[35, 83], percentage of bias[23, 74] and perseveration score[74] may be analyzed. Errors to acquisition criterion may be split into those committed in three distinct phases of learning - chance, early and late - which (in a session comprising 100 trials) may be defined as performance levels of ≤61%, 61-70% and 71-85%, respectively[23]. Errors to reversal criterion may also be split into those committed in distinct phases of learning, e.g., into prechance and above, which (in a session comprising 100 trials) may be defined as performance levels of ≤38%, and 39-85%, respectively[74]. Number of sessions required to complete the VMCL-specific pretraining phase may also be calculated. We refer the reader to General Considerations and PROCEDURE for further details.

### D) Autoshaping

Autoshaping in rats and mice may be conducted using Campden touchscreen chambers, which are suitably equipped (see MATERIALS)[8]. Previous work (in rats, but not mice) has also used bespoke apparatus built in-house at the University of Cambridge. During a trial, a white rectangular stimulus is presented on one side of the screen (the left or right) for a prespecified stimulus duration (standard: 10 s) (see Fig. 8). A stimulus on one side of the screen (e.g., left) is designated as the CS+ and the other as the CS−, counterbalanced across subjects. Upon CS+ offset a tone (1 s, 3 kHz) is emitted, a reward is delivered to the magazine, and the magazine is illuminated. Upon CS− offset, there is no tone or reward. Infrared photobeams in front of each side of the screen detect approaches to each side, and entries to the reward collection magazine are also detected. Following stimulus offset (and, if reward was delivered, entry into the magazine for reward collection), a variable ITI (standard range: 10-40 s) begins, after which the animal must break the infrared photobeam near the rear of the chamber (opposite the touchscreen) to initiate the next trial. Initiation is followed immediately by a click (0.2 s) and stimulus onset. This maximises the probability that the animal will be able to view both sides of the screen upon stimulus presentation and also minimises inadvertent stimulus approaches. The houselight is off throughout the task.

Each pair of trials is comprised of one CS+ trial and one CS− trial, such that each 40 trial session includes 20 presentations of each type.

Depending on the Autoshaping results obtained and hypotheses being tested, an “omission” probe phase may be performed to assess the nature of the associations governing responding. Sessions in this probe phase are identical to Autoshaping, except that approach to the CS+ prevents reward delivery. If the previously acquired autoshaping response is governed by a Pavlovian association, stimulus discrimination (as measured by approaches) should be resistant to reward omission across multiple sessions.

Autoshaping is preceded by Stage 1 of standard pretraining, and by a unique pretraining phase in which reward is delivered after a variable ITI (0-30 s; additional time allowed if necessary to ensure animal is not in magazine when ITI ends), with the magazine illuminated and a tone emitted upon delivery. The animal must enter the magazine to collect the reward (upon which the magazine light is extinguished) to initiate the next delay period.

The primary performance measures in this task are the number and latency of approaches to the CS+ and CS− side of the chamber. The number and latency of touches to the CS+ and CS− side of the screen are also recorded. Following initial chamber habituation and training, this task is acquired rapidly, with both control rats and mice displaying clear CS+/CS− discrimination within 5 daily sessions.

## MATERIALS

### REAGENTS

- Rats or mice (see REAGENT SETUP)
- Animal housing (see REAGENT SETUP)
- Rodent food pellets (e.g., Rodent Pellets, Special Diets Services, UK)
- Rewards: we use solid (e.g., Bio-Serv® purified rodent Dustless Precision Pellets®, 45 mg (rat)/14 mg (mouse), through Sandown Scientific, Esher, UK) or liquid (Yazoo® strawberry milkshake, FrieslandCampina UK Ltd) food reward

**Caution** When filling reward dispenser with Dustless Precision Pellets®, take care to discard dust, as this can potentially clog dispensers.

**Caution** All liquid reward containers and delivery lines should be thoroughly rinsed at the end of each testing day to prevent clogging and/or growth of potentially harmful micro-organisms.

- Cleaning materials (e.g., TriGene®, 70% ethanol solution, stiff brush)

### EQUIPMENT

- Sound- and light-attenuating box with ventilation system, enclosing an operant chamber and reward delivery system.
- Touchscreen operant chambers (from, e.g., Campden Instruments Ltd., Med Associates Inc., other commercial suppliers; or custom-made operant system). Note that these are species-specific. See EQUIPMENT SETUP.
- Camera above chamber, connected to closed circuit monitor and digital video recording device, to monitor and record animals’ behavior (optional but recommended)

- Controlling software and devices (generally available from operant chamber supplier)
- Black plastic masks with response windows (the number and size of which differ between tasks – see Table 1 and Fig. 9)
- Shelf for rat chamber (for some tasks, see EQUIPMENT SETUP)
- Appropriate data analysis software
- Personal protection equipment (e.g., disposable medical gloves, lab coat or coverall, FFP2 mask) should always be worn when handling or working near animals, to minimize allergen exposure.

## REAGENT SETUP

**Rodents—**Laboratory-bred or commercially available rats/mice are generally used for testing. There are some advantages to testing male rodents, such as avoiding potential estrus cycle-related performance variability in females[119, 120], and potentially increased inter-male aggression when males must be tested in the same apparatus as females. Most commonly, we use Lister Hooded rats and mice on the C57BL/6 or 129 substrain genetic backgrounds, and we prefer to begin training when rodents are young adults, e.g., mice 10-14 weeks old. However, females[51, 55, 57, 58, 60], aged rodents[12] and various strains (see, for example[16, 49, 52, 121]) have been tested. Choice of animals is an important consideration for all cognitive and behavioral testing of rodents.

**Caution** All experiments using live animals must be approved by national and institutional bodies, and performed according to their regulations.

**Caution** If animals are not fully grown when food restriction begins, they must be allowed to gain sufficient weight as they continue to grow. Standard strain growth curves are available for guidance (e.g., http://jaxmice.jax.org/support/weight/index.html).

**Animal housing—**Rats and mice should usually be housed in groups (e.g., 2-5), with sawdust, bedding and (optional, although recommended) shelter (or alternatives). Cages, bedding and so on should be cleaned or changed weekly. The housing room should be maintained at a constant temperature (21 ± 2 °C) and humidity (55 ± 10%). Lighting is usually on a 12-hour light-dark cycle, with lights off at 7:00 AM or 7:00 PM. We favor lights off at 7:00 AM, so that rodents can be tested in the active period of their circadian cycle. To our knowledge, conducting behavioral testing during the dark phase of an inverse light cycle has no adverse effect on the welfare of mice, but may improve activity levels, learning and memory[122-124]. However, researchers should be aware that lighting phase could potentially interact with sex, strain, experimental manipulations, etc. to influence performance. When shifting or inverting the light cycle of rodents, allow sufficient time for rodents to become fully entrained to the inverse cycle before commencing behavioral testing (see, e.g.,[125]). We tend to allow one day per hour of shift. This, of course, is an important consideration for all cognitive and behavioral testing of rodents.

## EQUIPMENT SETUP

**Rodent touchscreen operant chambers—**Rodent touchscreen operant chambers made by different companies may vary, but share many common features. The specific model used depends on the experimenter's needs and preference. Here, we describe mouse and rat chambers from Campden Instruments Ltd. and our in-house assembled boxes.

**Campden chambers:** Housed inside a dense fiberboard box, these are equipped with a fan (for ventilation and masking extraneous noise), touchscreen monitor (rat: 15.0 inch, screen resolution 1024 × 768 (rotated); mouse: 12.1 inch, screen resolution 600 × 800), tone and click generator, houselight (LED), magazine unit (with light and infrared beam to detect entries; in the standard configuration this is outside the testing arena, on the wall opposite the touchscreen) and pellet dispenser and/or pump connected to bottles of liquid reward (see Fig. 10 for rat chamber). The chambers have a trapezoidal shape (rat: 30 h × 33 l (screen-magazine) × 25 w (at screen) or 13 w (at magazine) cm; mouse 20 h × 18 l × 24 or 6 w cm), composed of 3 black plastic walls opening on to the touchscreen, intended to help focus the animal's attention to the touchscreen and reward delivery area. The touchscreen uses infrared photocells, and therefore does not require the subject to exert any pressure in order for responses to be registered. Our experience is that rodents work most readily and learn fastest with these IR beams, and not when they have to exert any pressure on the screen, although we have not done a properly controlled experiment to test this idea. We typically observe rodents responding to the screen with their noses (see Supplementary Video 1). Access to the chamber is through a transparent lid, which can be secured to the trapezoidal walls with latches during animal testing. The floor consists of perforated stainless steel, raised above a tray lined with filter paper. Two additional photobeams extend between the side walls of the arena, parallel to the screen, to detect the movement of an animal in the front (rat: ~6 cm from the screen; mouse: ~7 cm) or the rear (rat: ~5 cm from the magazine; mouse: ~3.5 cm) parts of the arena. A small infrared camera can be installed above the chamber to monitor animals' behavior (optional, but recommended). In rat chambers, attaching a shelf to the mask has proved to be effective at reducing impulsive responses and improving attention directed to the stimuli, by forcing the rat to rear up before making a choice[2]. In Campden rat chambers, a spring-hinged "shelf" (24 w × 6 l cm) can be attached 15 cm above the floor at a 90° angle to the screen and mask. Our laboratory uses these shelves for rats in the majority of tasks (the exception in this paper being Autoshaping). Campden Instruments Ltd. provide advice on setting up the touchscreen equipment, including touchscreen and reward dispenser calibration.

**Our in-house chambers:** Housed inside a melamine box, chambers (modified in our lab from Med Associates operant chambers) are equipped with a fan, infrared touchscreen monitor (rat: 29.0 h × 23.0 w cm; mouse: 16.0 h × 21.2 w cm; Craft Data Limited, Chesham, UK), tone generator, click generator, houselight (~3 W), magazine and pellet dispenser. The touchscreen does not require the subject to exert any pressure in order for touches to be registered. The chambers have a rectangular shape, consisting of a metal frame with clear Perspex walls (rat: 29 h × 31 l × 24 w cm; mouse: 13 h × 25 l × 19 w cm; excluding space below floor). Access is through a hinged side wall, secured with a latch during testing. The floor consists of stainless steel bars spaced 1 cm apart, above a tray lined with filter paper. The magazine is equipped with a light and a photocell nose poke detector. A spring-hinged "shelf" (20.5 w × 6 l cm) is also fitted in rat chambers 14.0 cm above the floor, at a 90° angle to the screen and mask.

**Masks and stimuli:** A black plastic mask (rat in-house: 38.7 h × 30.0 w cm; rat Campden: 35.8 h × 28.0 w cm; mouse in-house: 11.8 h × 22.8 w cm; mouse Campden: 24.3 h × 28.0 w cm) with response windows is fitted in front of the touchscreen to reduce accidental screen touches and make response locations clearly identifiable from the background. These have varying numbers and sizes of response windows, depending on the task (see Table 1).

**Autoshaping:** As far as we know, this task can only be run in the Campden chambers described above at the present time. In contrast to the usual chamber configuration, the reward collection magazine unit is positioned immediately in front of the centre of the

touchscreen, inside the arena (see Fig. 9b). The photobeam which usually traverses the width of the chamber in front of the screen is split into two independent beams by the magazine, such that approaches to each side of the screen can be measured separately. An additional photobeam traverses the side of the box opposite the screen as in the normal setup.

**Caution** When the apparatus is used in the "Autoshaping configuration", a fitted cover must be used to seal the hole in the chamber walls that usually allows access to the externally located magazine. Ensure the magazine is secured correctly to prevent possible injury to subject in the arena. Also ensure that the infrared beam microswitch is set to the "Autoshaping configuration" (as explained in manufacturer's manual).

**Controlling software and devices—**Controlling software can be purchased from the suppliers of the operant chambers, e.g., "Whisker Server"[126]; ELO software (ELO Touchsystems Inc.). Multiple chambers may be controlled by a single computer, although it is important to check that minimum system requirements are met (e.g., memory and graphics cards) to prevent delays in stimuli presentation and chamber responses. All task software is based on earlier publications and is available (excluding, in some cases, recent modifications) from Campden Instruments Ltd., and in some cases from Med Associates Inc. (K-Limbic) or other suppliers. Alternatively, software may be programmed using common programming languages, e.g., Visual Basic 6.0 (Microsoft, Redmond, WA).

## PROCEDURE

### Preparation for pretraining

**1** If it is not necessary to transport animals to the facility from an external source, proceed directly to Step 2 of the protocol. If transportation is necessary, conduct this, followed by an acclimatization period of 7 days (minimum). During this 7 days, provide animals with *ad libitum* food and water, and conduct no procedures. You may begin handling and weighing the animals after 2 days of acclimatization. Proceed to Step 3 after the 7 day acclimatization period.

**Critical step** We advise consulting with your institutional animal care regulatory body when planning and designing experiments, regarding matters including food restriction and housing.

**Critical step** Some cohorts of mice have relatively high between-subject variability, and so larger Ns are required. There are many variables that can affect variability, such as strain, maternal care, events during transportation, etc.. We advise minimising the age range of cohorts to reduce potential age-related variability. Where possible, calculation of Ns should be based on a power calculation that is based on previous work with that strain of animal, ideally from the same supplier. This of course is an important consideration for all cognitive and behavioral testing of rodents.

**Critical step** Train all animals using this preparation and pretraining (Steps 1-9) prior to their first instrumental touchscreen task (Steps 10A, B or C). If subjects have previously been trained and tested on another instrumental touchscreen task in the battery, maintain food restriction and start at pretraining Step 9. For pretraining prior to Autoshaping (Step 10D), proceed to Step 10D after Steps 1-5. As discussed in EXPERIMENTAL DESIGN, touchscreen tasks (e.g., 10A-C, also see[4, 5]) may be employed in flexible combinations and orders.

**2** Weigh each animal for three consecutive days with *ad libitum* food and water and calculate the mean free-feeding weight of each animal.

**Critical step** Ensure that each animal can be reliably identified.

**3** Begin food restriction, adhering to all relevant institutional and national guidelines. Slowly reduce (e.g., over 3-7 d) the weight of individual animals down to the goal weight, which will be a percentage of the measured free-feeding weight (e.g., we use 85-95%, which is in-line with our institutional and national guidelines) by controlling the daily amount of food they are given (e.g., for rats, ~7 g food per 100 g body weight; for mice, ~2-3 g food per 25-35g mouse). Start Step 4 when animals are close to their goal weights. Maintain food restriction throughout touchscreen testing.

**Critical step** It is important to check the weight of animals daily (mice) or twice a week (rats) until the target weight is reached. This also helps habituate the animals to being handled. Aim to avoid weight reduction of greater than 5% per day, and weight reduction below 85% of free-feeding.

**4** Introduce reward (pellets or milkshake) inside the cage to habituate the animals for 1-3 days. Solid rewards may be scattered on the cage floor; liquid rewards should be put into a shallow, wide-based dish.

## Pretraining

**5** Set up the apparatus (see MATERIALS) for this pretraining stage (Stage 1), with all electronic components on so that subjects may habituate to these. Here and in all subsequent steps, use touchscreen masks and stimuli as appropriate for the task (e.g., Visual Discrimination; see EXPERIMENTAL DESIGN, MATERIALS and Table 1). Note that for the VMCL task, only locations 1 (left-most) and 3 (right-most) should be used during pretraining Steps 7-9. It is not necessary to run any software during Stage 1, but we recommend recording subjects' activity if the necessary apparatus and software is available (e.g., chambers from Campden Instruments Ltd). Place ~10 reward pellets or 0.2 ml liquid reward in the magazine of each chamber (if the computer program you are using does not do this automatically). Place each rodent in its assigned chamber for 30 min. Remove the rodent and check that reward has been consumed. Return each animal to their respective home cage. Test all subjects on Stage 1 for at least two sessions. The criterion for advancing to the next Step is consuming all rewards in a session.

**Critical step** Animals require fewer standard rodent food pellets when receiving rewards during training; adjust daily food allowance as appropriate to maintain goal weight.

**Critical step** Aim to train, weigh and feed each animal at approximately the same time each day and use the same operant box for each animal during training. Always counterbalance chambers and testing times across experimental groups. It is good practice to weigh mice daily, but once or twice per week may be sufficient for rats. We recommend one session per day, 5-7 days per week.

**Critical step** Advance individual subjects to the next pretraining stage when they reach criterion, even if some animals in the group remain on previous stage(s).

**Critical step** Operant chambers should be cleaned regularly (e.g., once a week, or more) to avoid context change during sensitive task phases, to ensure the touchscreen and infrared photobeams retain maximum sensitivity, and to prevent accumulation of dirt and excrement.

We typically dismantle inner chambers (as far as possible), and clean with surface disinfectants (e.g., TriGene® and 70% ethanol) using paper towel or a stiff brush.

**6** Set up the apparatus as detailed in MATERIALS and the software program for this stage (Stage 2) with settings as detailed in EXPERIMENTAL DESIGN. Place each subject in its assigned chamber, and start the session. The session finishes after 60 min or 100 trials (rat)/ 30 trials (mouse) are completed (whichever comes first). (For pretraining prior to mouse Object-Location Paired-Associates Learning, there are 36 trials per session.) After session termination, return each animal to their respective home cage. Advance individual subjects to the next training phase when they achieve a criterion of completing all trials (mice) or 60 trials (rats) within 60 min.

**Critical step** At the end of each session, record critical data for each subject (e.g., number of correct responses, number of trials completed), in case of computer malfunction. However, most software programs will log many other measures (see EXPERIMENTAL DESIGN).

**Critical step** If testing the effects of a manipulation conducted before onset of the experiment (Case 1, see EXPERIMENTAL DESIGN), ensure that animals in experimental and control groups complete comparable numbers of trials per session. Cap the number of trials given per session to accommodate the lowest responders.

**7** Repeat Step 6/Stage 2 for Stage 3 using the appropriate software program (see EXPERIMENTAL DESIGN).

**8** Provide a single free reward (if your program does not do this automatically) for Stage 4. Otherwise, proceed as in Step 6/Stage 2, using the appropriate software program (see EXPERIMENTAL DESIGN).

**9** Proceed as in Step 8/Stage 4 for stage 5, using the appropriate software program (see EXPERIMENTAL DESIGN). The criterion for completing this stage is completing all trials with ≥ 80% correct (not including CTs) within 60 min (rat), or with ≥ 75% correct within 35 min (mouse), on 2 consecutive sessions. (Allow 40 min for mice in pretraining for Object-Location Paired-Associates Learning, in which mice receive 36 trials per session.)

**Critical step** There is likely to be variation in the number of days that animals require to complete pretraining. We suggest “resting” animals when they reach criterion, with “reminder” sessions, then rebaselining all subjects so that the entire group can advance to a specific touchscreen task on the same day (see EXPERIMENTAL DESIGN). If subjects are scheduled to receive experimental treatments after pretraining but prior to task acquisition (Case 2, see EXPERIMENTAL DESIGN), perform these now (after Step 9), counterbalancing control and experimental groups according to the number of sessions required to complete pretraining, then rebaseline on Step 9/Stage 5 before task-specific training.

## Task

**10** Proceed to Visual Discrimination (Option A), Object-Location Paired-Associates Learning (Option B), VMCL (Option C; rats only) or Autoshaping (Option D).

### A) VISUAL DISCRIMINATION—

**i *Visual Discrimination acquisition training*** When subjects are ready for task training to begin, counterbalance stimulus reward contingencies (such that approximately half of each

group receive Stimulus A as CS+ and B as CS−, and the rest the reverse), according to the number of sessions required to complete pretraining.

**ii** Begin training on once-daily sessions of Visual Discrimination acquisition, 5-7 days per week. Provide a single free reward (if your program does not do this automatically). Set up the apparatus as detailed for this task in MATERIALS and the software program for this stage with settings as detailed in EXPERIMENTAL DESIGN with reward contingency as appropriate for each subject. Place each subject in its assigned chamber, and start the session. The session finishes either after 60 min or 100 trials (rat)/30 trials (mouse) are completed (whichever comes first). After session termination, return each animal to their respective home cage.

**Critical step** Give careful consideration to the stimulus set you choose (see, e.g., Fig. 3a-d). Standard rat stimuli ("Spider" and "Plane") are also standard stimuli used in the Object-Location Paired-Associates Learning task, so should be avoided here if rats have previously been, or may subsequently be, tested on Object-Location Paired-Associates Learning. If you wish to use morphed stimuli as a post-training manipulation, this may also affect your initial choice of stimuli.

**Critical step** Carefully monitor visual stimulus biases on the first day of testing (see Data analysis, Step v). If animals show strong stimulus biases, consider revising the stimuli. This of course is an important consideration for all cognitive and behavioral testing of rodents involving object discriminations.

**Critical step** Given that performance is likely to be poor at the start of training, with animals therefore receiving many CTs, limit sessions to 50 trials (rat)/15 trials (mouse) in 60 min, for at least 2 sessions. Continue until subjects can complete this in 30 min. Give each subject an even number of these reduced sessions, such that they can be combined into full 100 (or 30)-trial sessions for analysis. If the subject completes fewer trials than required, the missed trials may be added on to the trials required in the next session (if less than ~10), or given in a new session.

**Critical step** If testing the effects of a manipulation conducted before onset of task acquisition (e.g., Cases 1 and 2, see EXPERIMENTAL DESIGN), ensure that animals in experimental and control groups complete comparable numbers of trials per session throughout task acquisition. Cap the number of trials given per session to accommodate the lowest responders.

**Critical step** At the end of each session, record critical data for each subject (e.g., number of correct responses, number of trials completed), in case of computer malfunction. However, most software programs will log many other measures (see EXPERIMENTAL DESIGN).

**iii** Continue training on once-daily sessions of Visual Discrimination acquisition, 5-7 days per week, until animals have reached the acquisition criterion for this task, at which point proceed to the next step. The acquisition criterion for this task is the completion of all trials with an accuracy of ≥ 80%, or alternatively, 85% (e.g.,[13, 51, 58]) (excluding CTs) for two consecutive sessions. If testing animals that received experimental manipulations prior to task acquisition (Cases 1 and 2, see EXPERIMENTAL DESIGN), the main experimental read-out may be differences in the rate of acquisition (and/or final performance level). If so, continue training all animals on all sessions, either for a given number of sessions (to allow plotting of an acquisition curve), or until the control and/or experimental group(s) attain criterion or stable performance. Alternatively, if post-training behavioral challenges are to follow (e.g., morphed visual stimuli, retention and reversal; see Step iv), and/or when post-

acquisition manipulations are to be conducted (e.g., Cases 3 and 4 in combination with continued training at asymptotic performance level, see EXPERIMENTAL DESIGN), we suggest “resting” animals when they reach criterion, with “reminder” sessions, until the entire group has achieved criterion, upon which the entire group may be rebaselined before progressing to Step iv (see EXPERIMENTAL DESIGN for details and alternatives). If subjects are scheduled to receive experimental treatments after acquisition but before Step iv (Case 3, see EXPERIMENTAL DESIGN), perform these when all animals have reached criterion (at least) once, counterbalancing control and experimental groups according to acquisition performance, then rebaseline.

**Critical step** In case of investigating the effects of post-acquisition treatment (Case 3 or 4) on behavioral challenges using stimuli that are not part of regular task acquisition (e.g., morphed stimuli, Step iv), animals should also be briefly (e.g., for one or two sessions) exposed to these before treatment to avoid confounds due to novelty or contextual change, and to allow for a within-subject pre- and post-treatment comparison of performance level. In the case of microinfusion studies, animals should be rebaselined after surgery until a stable performance level is reached, and introduced to the relevant novel stimuli at this point. Before commencing subsequent vehicle and drug infusion, a mock infusion involving the insertion of the infusion cannula only should be performed, followed by a vehicle infusion to assess non-specific effects on performance.

**iv *Post-training experimental manipulations***. Depending on the aims of the experiment, proceed with appropriate post-training manipulations. Various posttraining manipulations are possible. For **continued training at asymptotic performance level**, conduct the experiment as in Step ii. Transient treatments may be performed in an appropriately controlled way (e.g., Case 4, Latin square design). For the **morphed visual stimuli** probe, conduct the experiment as in Step ii, using stimuli that are morphed (or blended) versions of those used in Step ii. Transient treatments may be performed in an appropriately controlled way (e.g., Case 4, Latin square design). For **Visual Discrimination retention**, rebaseline all subjects together when they have all reached criterion (at least) once, and begin a retention interval (e.g., 7-10 days; exactly the same for each subject). Then test animals as in Step ii. It may be sufficient to test for a certain number of sessions (e.g., 5, 10) to assess retention, rather than testing until criterion is reattained. For **reversal**, reverse the reward contingencies, i.e. S+ becomes S−, and vice versa. For details, see 5.

**v *Data analysis***. Analyze performance measures for each Visual Discrimination phase (see EXPERIMENTAL DESIGN):

- Number of sessions required to complete pretraining (Steps 5-9), and/or individual pretraining Steps.
- Percentage accuracy (in the form of an acquisition curve, if all subjects complete a certain minimum number of sessions, e.g., 5, 10)
- Sessions, trials and errors required to reach criterion.
- Average correct and incorrect response latency.
- Average magazine latency.
- Percentage of bias (particularly in the first session for each animal).
- Perseveration score.

## B) OBJECT-LOCATION PAIRED-ASSOCIATES LEARNING—

**i *Object-Location Paired-Associates Learning training***. Begin training on once-daily sessions of Object-Location Paired-Associates Learning, 5-7 days per week. Provide a single free reward (if your program does not do this automatically). Set up the apparatus as detailed for this task in MATERIALS and the software program for this stage with settings as detailed in EXPERIMENTAL DESIGN. Place each subject in its assigned chamber, and start the session. Finish the session either after 60 min or 90 trials (rat)/36 trials (mouse) are completed (whichever comes first). After session termination, return each animal to their respective home cage.

**Critical step** Given that performance will be poor at the start of training, with animals therefore receiving many CTs, limit sessions to 45 trials (rat)/18 trials (mouse) in 60 min. Continue until subjects can complete this in 30 min. Give each subject an even number of these reduced sessions, such that they can be combined into full 90 (or 36)-trial sessions for analysis. If the subject completes fewer trials than required, the missed trials may be added on to the trials required in the next session (if less than ~10), or given in a new session. When giving a reduced number of trials per session, ensure that an equal number of each trial type is presented.

**Critical step** If testing the effects of a manipulation conducted before onset of the experiment or task acquisition (Cases 1 and 2, see EXPERIMENTAL DESIGN), ensure that animals in experimental and control groups complete comparable numbers of trials per session throughout task acquisition. Cap the number of trials given per session to accommodate the lowest responders.

**Critical step** At the end of each session, record critical data for each subject (e.g., number of correct responses, number of trials completed), in case of computer malfunction. However, most software programs will log many other measures (see Data analysis).

**ii** Continue training on once-daily sessions of Object-Location Paired-Associates Learning, 5-7 days per week until animals have reached the acquisition criterion for this task, at which point proceed to the next step. The acquisition criterion for this task is the completion of all trials with an accuracy of ≥ 80% (excluding CTs) for two consecutive sessions. If testing animals that received experimental manipulations prior to task acquisition (Cases 1 and 2, see EXPERIMENTAL DESIGN), the main experimental read-out will likely be differences in the rate of acquisition (and/or final performance level). Therefore, continue training all animals on all sessions, either for a given number of sessions (to allow plotting of an acquisition curve), or until the control and/or experimental group(s) attain criterion or stable performance. All animals may then progress to the same-object probe (Step iii; if required) on the same day. Alternatively, when post-acquisition manipulations are to be conducted (e.g., Cases 3 and 4, in combination with continued training at asymptotic performance level, see EXPERIMENTAL DESIGN), we suggest “resting” animals when they reach criterion, with “reminder” sessions, until the entire group has achieved criterion, upon which the entire group may be rebaselined before progressing to Step iii (see EXPERIMENTAL DESIGN for details and alternatives). If subjects are scheduled to receive experimental treatments after acquisition but before Step iii (Case 3, see EXPERIMENTAL DESIGN), perform these when all animals have reached criterion (at least) once, counterbalancing control and experimental groups according to acquisition performance, and then rebaseline.

**Critical step** The performance of mice on this task is less reliable than that of rats[8, 45, 54, 56, 60], and will depend on strain, age, etc. It may be necessary to apply a less strict performance criterion (e.g., 70%) for some strains of mice.

**Critical step** In the case of microinfusion studies, animals should be rebaselined after surgery until a stable performance level is reached. Before commencing subsequent vehicle and drug infusion, a mock infusion involving the insertion of the infusion cannula only should be performed, followed by a vehicle infusion to assess non-specific effects on performance.

**iii *Post-training experimental manipulations***. Depending on the aims of the experiment, perform appropriate post-training manipulations. Various post-training manipulations are possible. For **continued training at asymptotic performance level**, conduct the experiment as in Step i. Transient treatments may be performed in an appropriately controlled way (e.g., Case 4, Latin square design). The same-object probe may be conducted subsequently, if required. For the **same-object probe**, proceed as in Step i, but using a modified software program (as detailed in EXPERIMENTAL DESIGN). Test animals for two sessions (once daily) of the same-object probe.

**Critical step** Avoid running the same-object probe prior to animals having reached criterion as exposure to the probe at this stage may encourage a visuomotor conditioning response (e.g., “see object 1 in any location, respond to location 1”) rather than the formation of an object-location association.

**iv *Data analysis***. Analyze the following behavioral variables (across acquisition, at performance asymptote and/or during the same-object probe, as appropriate for your study; see EXPERIMENTAL DESIGN):

- Number of sessions required to complete pretraining (Steps 5-9), and/or individual pretraining Steps.
- Percentage accuracy (in the form of an acquisition curve, if all subjects complete a certain minimum number of sessions, e.g., 30).
- Sessions, trials and errors required to reach criterion.
- Average correct and incorrect response latency.
- Average magazine latency.
- Percentage of bias.
- Perseveration score.
- Trial type analysis (percentage accuracy for each of the six trial types individually).

### C) VMCL—

**i *VMCL-specific pretraining***. Provide a single free reward (if your program does not do this automatically). Set up the apparatus as detailed for this task in MATERIALS and the software program for this stage with settings as detailed in EXPERIMENTAL DESIGN. Place each subject in its assigned chamber, and start the session. The session usually finishes either after 60 min or 100 trials are completed (whichever comes first). After session termination, return each animal to their respective home cage. Continue training each animal once-daily, 5-7 days per week, until it reaches a criterion of at least 80% of (non-correction) trials correct (and all trials completed) in a 2 consecutive sessions, with LH 2 s. There is likely to be little variation in the number of days that animals require to complete VMCL-specific pretraining, but if there is a difference of 2 or more days between the fastest and slowest subjects, we suggest “resting” animals when they reach criterion, with “reminder” sessions, then rebaselining the group so that the entire group can advance to VMCL training on the same day (see EXPERIMENTAL DESIGN). If subjects are scheduled to receive

experimental treatments after pretraining but prior to task acquisition (Case 2, see EXPERIMENTAL DESIGN), these may be performed now (instead of after Step 9), counterbalancing control and experimental groups according to the number of sessions required to complete VMCL-specific pretraining, then subjects rebaselined before task-specific training.

**Critical step** At the end of each session, record critical data for each subject (e.g., number of correct responses, number of trials completed), in case of computer malfunction. However, most software programs will log many other measures (see Data analysis).

**Critical step** If individual subjects have difficulty with the 2 s LH, use a longer LH (e.g., 5 s) in the first instance, and then gradually reduce as appropriate (based on the subject's reaction time).

**Critical step** If testing the effects of a manipulation conducted before onset of the experiment or task acquisition (e.g., Case 1, see EXPERIMENTAL DESIGN), ensure that animals in experimental and control groups complete comparable numbers of trials per session throughout VMCL-specific pretraining. Cap the number of trials given per session to accommodate the lowest responders.

**ii *VMCL training***. When all subjects have completed Step i, assign animals (of each experimental condition) to two groups counterbalanced according to the number of sessions required to achieve criterion for Step i. For one group, Stimulus A will indicate that a response to the left location is correct (and right incorrect), and Stimulus B will indicate the opposite. These contingencies will be reversed for the other group.

**iii** Begin VMCL training trials. Provide a single free reward (if your program does not do this automatically). Set up the apparatus as detailed for this task in MATERIALS and the software program for this stage with settings as detailed in EXPERIMENTAL DESIGN with reward contingency as appropriate for each subject. Place each subject in its assigned chamber, and start the session. The session usually finishes either after 60 min or 100 trials are completed (whichever comes first). After session termination, return each animal to their respective home cage. Train once-daily, 5-7 days per week.

**Critical step** Given that performance will be poor at the start of training, with animals therefore receiving many CTs, limit sessions to 50 trials in 60 min, for at least 2 sessions. Continue until subjects can complete this in 30 min. Give each subject an even number of these reduced sessions, such that they can be combined into full 100-trial sessions for analysis. If the subject completes fewer trials than required, the missed trials may be added on to the trials required in the next session (if less than ~10), or given in a new session.

**Critical step** If testing the effects of a manipulation conducted before onset of the experiment or task acquisition (Cases 1 and 2, see EXPERIMENTAL DESIGN), ensure that animals in experimental and control groups complete comparable numbers of trials per session throughout VMCL acquisition. Cap the number of trials given per session to accommodate the lowest responders.

**Critical step** Analyze the data after the first session, checking that no subject has a significant side bias (see Data analysis, Step vi).

**iv** Continue training on once-daily sessions of VMCL, 5-7 days per week, until animals have reached the acquisition criterion for this task, at which point proceed to the next step. The acquisition criterion for this task is the completion of all trials with an accuracy of ≥ 85% (excluding CTs) for two consecutive sessions. If testing animals that received experimental

manipulations prior to task acquisition (Cases 1 and 2, see EXPERIMENTAL DESIGN), the main experimental read-out will likely be differences in the rate of acquisition (and/or final performance level). If so, continue training all animals on all sessions, either for a given number of sessions (to allow plotting of an acquisition curve), or until the control and/or experimental group(s) attain criterion or stable performance. Alternatively, if post-training behavioral challenges (i.e. reversal, Step v) are of interest, and/or when post-acquisition manipulations are to be conducted (e.g. Cases 3 and 4, in combination with continued training at asymptotic performance level, see EXPERIMENTAL DESIGN), we suggest "resting" animals when they reach criterion, with "reminder" sessions, until the entire group has achieved criterion, upon which the entire group may be rebaselined before progressing to Step v (see EXPERIMENTAL DESIGN for details and alternatives). If subjects are scheduled to receive experimental treatments after acquisition but before Step v (Case 3, see EXPERIMENTAL DESIGN), perform these when all animals have reached criterion (at least) once, counterbalancing control and experimental groups according to acquisition performance, and then rebaseline.

**Critical step** In the case of microinfusion studies, animals should be rebaselined until a stable performance level is reached, after surgery. Before commencing subsequent vehicle and drug infusion, a mock infusion involving the insertion of the infusion cannula only should be performed, followed by a vehicle infusion to assess non-specific effects on performance.

**v *Post-training experimental manipulations***. Depending on the aims of the experiment, perform appropriate post-training manipulations. Various post-training manipulations are possible. For **continued training at asymptotic performance level**, conduct the experiment as in Step iii. Transient treatments may be performed in an appropriately controlled way (e.g., Case 4, Latin square design). For **reversal**, proceed as in Step iii, but with modifications to the program. Continue training until subjects reattain the criterion (85% of trials correct on 2 consecutive days), or for a fixed number of sessions.

**vi *Data analysis***. Analyze performance measures for each VMCL phase (see EXPERIMENTAL DESIGN):

- Number of sessions required to complete pretraining (Steps 5-9), and/or individual pretraining Steps.
- Percentage accuracy (in the form of an acquisition curve, if all subjects complete a certain minimum number of sessions, e.g., 5, 10)
- Sessions, trials and errors required to reach criterion. Note that errors may be split into those committed when the animal is performing below chance, at chance, and/or above chance, as appropriate (see EXPERIMENTAL DESIGN).
- Average correct and incorrect response latency.
- Average magazine latency.
- Percentage of bias (particularly in the first session for each animal).
- Perseveration score.

## D) AUTOSHAPING—

**i *Autoshaping pretraining***. Begin testing all subjects on the same day. Set up the apparatus as detailed for this task in MATERIALS and the software program for this stage with settings as detailed in EXPERIMENTAL DESIGN. Place each subject in its assigned chamber, and start the session. Finish the session either after 60 min or 40 trials are

completed (whichever comes first). After session termination, return each animal to their respective home cage. Train subjects once daily, 5-7 days per week. Criterion for this stage is completing all trials in the allotted time, with all rewards consumed. There is likely to be little variation in the number of days that animals require to complete this pretraining, but if there is a difference of 3 or more days between the fastest and slowest subjects, we suggest "resting" animals when they reach criterion, with "reminder" sessions (see EXPERIMENTAL DESIGN for details and alternatives). The group may then be rebaselined together before advancing to Autoshaping training on the same day. If subjects are scheduled to receive experimental treatments after pretraining but prior to task acquisition (Case 2, see EXPERIMENTAL DESIGN), perform these now, counterbalancing control and experimental groups according to the number of sessions required to complete pretraining, then rebaseline before task-specific training.

**Critical step** The autoshaping process may leave animals with a side bias, even if the omission probe step is run. Therefore, we do not recommend that animals are tested on any other task after Autoshaping. Animals need not necessarily be naive for this task, but we recommend that they are, because the associations formed in instrumental touchscreen tasks could possibly interfere with autoshaping.

**Critical step** At the end of each session, record critical data for each subject (e.g., number of trials completed, number of approaches to left and right sides), in case of computer malfunction. However, most software programs will log many other measures (see Data analysis).

**Critical step** If testing the effects of a manipulation conducted before onset of the experiment (Case 1, see EXPERIMENTAL DESIGN), ensure that animals in experimental and control groups complete comparable numbers of trials per session. Cap the number of trials given per session to accommodate the lowest responders.

**ii *Autoshaping acquisition training***. Divide animals (of each experimental condition) into "CS+ left side" and "CS+ right side" groups. Counterbalance these according to the number of sessions required to complete Autoshaping pretraining (Step i).

**iii** Set up the apparatus as detailed for this task in MATERIALS and the software program for this stage with settings as detailed in EXPERIMENTAL DESIGN with reward contingency as appropriate for each subject. Place each subject in its assigned chamber, and start the session. The session finishes either after 90 min or 40 trials are completed (whichever comes first). After session termination, return each animal to their respective home cage. Test all subjects for a minimum of 2 sessions, once-daily, 5-7 days per week.

**Critical step** Data should be analyzed on a daily basis to monitor performance (see Data analysis, Step vi). Animals should begin to discriminate between the CS+ and CS−, as measured by the number of approaches to the two stimulus locations, within approximately 4-5 sessions. The latency to approach each location upon stimulus display should also indicate discriminative performance.

**Critical step** If testing the effects of a manipulation conducted before onset of the experiment or task acquisition (Cases 1 and 2, see EXPERIMENTAL DESIGN), ensure that animals in experimental and control groups complete comparable numbers of trials per session throughout task acquisition. Cap the number of trials given per session to accommodate the lowest responders.

**iv** Continue training all subjects until discriminated approach is clearly evident in the control group, regardless of experimental manipulation (Cases 1-4, see EXPERIMENTAL

DESIGN). If testing animals that received experimental manipulations prior to task acquisition (Cases 1 and 2, see EXPERIMENTAL DESIGN), the main experimental read-out will likely be differences in the rate of acquisition (and/or final performance level). If subjects are scheduled to receive experimental treatments after acquisition but before Step v (e.g., Case 3, in combination with continued training at asymptotic performance level, see EXPERIMENTAL DESIGN), perform these now, counterbalancing control and experimental groups according to acquisition performance. Before progressing to Step v, rebaseline all animals on acquisition training (see EXPERIMENTAL DESIGN) until performance of all subjects has been stable for at least two days.

**Critical step** In the case of microinfusion studies, animals should be rebaselined until a stable performance level is reached, after surgery. Before commencing subsequent vehicle and drug infusion, a mock infusion involving the insertion of the infusion cannula only should be performed, followed by a vehicle infusion to assess non-specific effects on performance.

**v** ***Post-training experimental manipulations***. Depending on the aims of the experiment, perform appropriate post-training manipulations. Various post-training manipulations are possible. For continued training at asymptotic performance level, conduct the experiment as in Step 10Diii. Transient treatments may be performed in an appropriately controlled way (e.g., Case 4, Latin square design). For the autoshaping omission probe, begin testing all subjects in the same session, on the day after their last Autoshaping session. Set up the apparatus as detailed for this task in MATERIALS and the software program for this stage with settings as detailed in EXPERIMENTAL DESIGN. Place each subject in its assigned chamber, and start the session. Finish the session either after 90 min or 40 trials are completed (whichever comes first). After session termination, return each animal to their respective home cage. Test all subjects for a minimum of 2 sessions, once-daily.

**vi** ***Data analysis***. Analyze performance measures from Autoshaping acquisition and omission sessions (see EXPERIMENTAL DESIGN):

- Number of sessions required to complete pretraining (Step 5) and Autoshaping pretraining (Step 10Di)
- Number of approaches made to the CS+ and CS− when displayed.
- Latency to approach each stimulus following onset.
- Number and latency of touches to each stimulus following onset.
- Latency to enter the magazine upon reward delivery.

## TIMING

Approximate timing for each step below is indicated as a number of sessions (i.e. days). As a rule, allow up to ~80 min per day per testing session from Step 5 onwards (or 110 min for steps 10Diii-v). These 80 minutes include 60 (or 90) min testing time, plus an additional 20 minutes for transporting animals from home- to testing-room, setting up software, etc. Cumulative time taken to test all animals in an experiment depends on the capacity to load multiple animals per test-run (i.e. number of chambers). Subsequent values for the number of days (sessions) it takes to execute these experiments typically reflect the approximate time it takes to test an average cohort of animals on each Step and are estimates based on our experience.

**Preparation for pretraining, Steps 1-4—**~6 or 10 days. Timing depends on whether animals are acquired from an external source, in which case a 7 day acclimatization period is

required, before onset of food restriction. After acclimatization, allow for approximately 3 days of initial food restriction before start of Stage 1 pretraining. Regular handling and weighing of animals can be started ~2 days after arrival. Reserve an average time per animal per day of ~5 min.

**Pretraining, Steps 5-9—**~10-15 sessions. Note that pretraining may take longer (e.g., ~10-30 sessions) when a mask with small response windows (e.g., < 3.0 × 3.0 cm) is used and/or if rebaselining is necessary. Also note that full pretraining is only necessary prior to the first instrumental task that an animal is tested on. Before subsequent instrumental tasks, animals should usually be tested on Step 9 only and, being well-trained, they may progress from this after only a few sessions.

**Visual discrimination acquisition, Steps 10Ai-iii—**The average number of sessions required to reach acquisition criterion with standard stimuli (e.g., Fig. 3a-c) is ~5-6 (rat)/ ~8-10 (mouse). Note that additional sessions may be required if “resting” and “rebaselining” are necessary (e.g., before Step iv).

**Visual discrimination post-training manipulations, Step 10Aiv—**Duration will depend on many factors, including experimental manipulation and performance. For retention, testing all animals for a predefined number of days (e.g., 5, 10) is likely to be sufficient.

**Object-Location Paired-Associated Learning, Steps 10Bi-ii—**As discussed in ANTICIPATED RESULTS, rats require an average of approximately 34 sessions to attain criterion of 80% correct, and mice approximately 50 sessions to attain a less stringent criterion (70%). Note that additional sessions may be required if “resting” and “rebaselining” are necessary (e.g., before Step iii).

**Object-Location Paired Associates Learning same-object probe, Step 10Biii—**2 sessions.

**VMCL-specific pretraining, Step 10Ci—**~2 sessions, dependent on performance.

**VMCL training, Step 10Cii-iv—**~8 sessions; see ANTICIPATED RESULTS.

**Autoshaping pretraining, Step 10Di—**~2 sessions.

**Autoshaping acquisition training, Steps 10Diii-iv—**~4-5 sessions until discrimination is clearly evident in the control group.

**Autoshaping omission probe, Step 10Dv—**~2 sessions.

## TROUBLESHOOTING

Consider excluding animals that fail to complete pretraining within a reasonable time frame (which may be determined *ad hoc* from the typical group performance). A drop-out rate of <10% is expected overall.

General troubleshooting advice can be found in Table 2. It is good practice to have spare light bulbs, touchscreen connector cables, infrared beam assemblies, touchscreens, pump tubing, and pellet dispensers available, because these components are particularly susceptible to failure. It is also important to check each test chamber at least once per week to ensure that infrared beams, light stimuli, and reward dispensers are functioning reliably.

Campden software includes programs that may be used to check the function of these basic components at the start of each day.

## ANTICIPATED RESULTS

### Visual Discrimination

Figure 11 shows a typical Visual Discrimination acquisition curve of Lister Hooded rats with photographic stimuli (see Fig. 3d) (C.A.O., unpublished results). These data are available in Supplementary Data 1.

### Object-Location Paired-Associates Learning

The typical results presented in this section are based on performance of male Lister Hooded rats (n =24; C.A.O., T.J.B., L.M.S., unpublished results) and male C57BL/6 mice on the Object-Location Paired-Associates Learning task[8, 45, 54, 56, 60], with data presented as mean ± SEM. Rats require an average of 2220 ± 184 trials or 34 ± 1.9 sessions to reach criterion (80% correct in 2 consecutive sessions). Mice require ~50 sessions (of 36 trials) to attain the less stringent criterion (70% correct in 2 consecutive days). Given the complexity of the task, it may occasionally (e.g., approximately 1 in 24 rats) be necessary to exclude poor performers which are statistical outliers, on an *ad hoc* basis. At maximum performance level (i.e. after all animals have reached 80% criterion), animals perform as follows, on an average example session: percentage correct 84.0% ± 1.0 (for rats; mice subjected to systemic saline treatment, n = 9[45]: ~80-85 %); number of CTs 19.5 ± 1.5 (mice: 9.55 ± 4.44); response latency to correct trials: 2.0 ± 0.10 s (mice: 6.38 ± 4.25 s); response latency to incorrect trials 2.1 ± 0.13 s (mice: 7.20 ± 4.35 s); average reward collection time 2.36 ± 0.04 s (mice: 2.36 ± 1.07 s)[45]. As for separate trial type analysis, we find that performance on particular trial types can be different for each animal, possibly depending on individual biases. Our rat task development data show that with the improved stimuli (see Fig. 5b) there is little initial *overall* bias towards particular trial types, with average performance across rats on the first day of training ranging from 41.5% ± 3.5 to 54.6% ± 3.9. In control animals, transfer to the same-object probe (in our hands) does not lead to a change in performance level (rats: standard task: 84.0% ± 1.0 to probe 82.6% ± 1.7), indicating that it is unlikely that animals rely on a configural strategy. Compared to rats, mice demonstrate (on average) minimal variation in performance of the six standard “Flower-Plane-Spider” trial types (A.E.H., L.M.S. & T.J.B., unpublished results).

## VMCL

Sham-lesioned rats tested in accordance with published versions of this protocol (without the VMCL-specific pretraining phase described here) took on average fewer than 10 sessions (of 60 trials) to reach the criterion (85% of trials correct on 2 consecutive days) for VMCL acquisition[74]. Using a near-identical reversal protocol to that presented here, sham lesioned rats required on average 10 sessions (of 60 trials) to reach the criterion (85% of trials correct on 2 consecutive days) for VMCL reversal[74].

### Autoshaping

Figure 12 shows the performance of *Dlg4*$^{-/-}$ and WT mice on the Autoshaping task as measured by the number of approaches to CS+ and CS−. Stimulus discrimination rapidly developed in WT animals, with CS+ approaches increasing and CS− approaches decreasing over 4 sessions[8]. Discrimination did not occur in the Dlg4−/− group[8]. In the WT group, the latency to approach the CS+ upon presentation also decreased between the first and fourth acquisition session, with no change in CS− approach latency[8]. No changes in approach latency to either stimulus were observed in the *Dlg4*$^{-/-}$ group[8] (latency data not shown).

To date our laboratory has not used the Autoshaping task with rats in the Campden apparatus. However, based on the similarity of performance of rats and mice in this task (regardless of apparatus), we believe that the timing of the various stages and the resultant data should not differ substantially from those presented here.

With regard to the reward omission probe, previous experiments in rats have shown that while the total number of stimulus approaches decreases, the elevated number of CS+ approaches, relative to CS− approaches is maintained[22]. This is consistent with the Pavlovian nature of the stimulus-reward association learned in the Autoshaping task.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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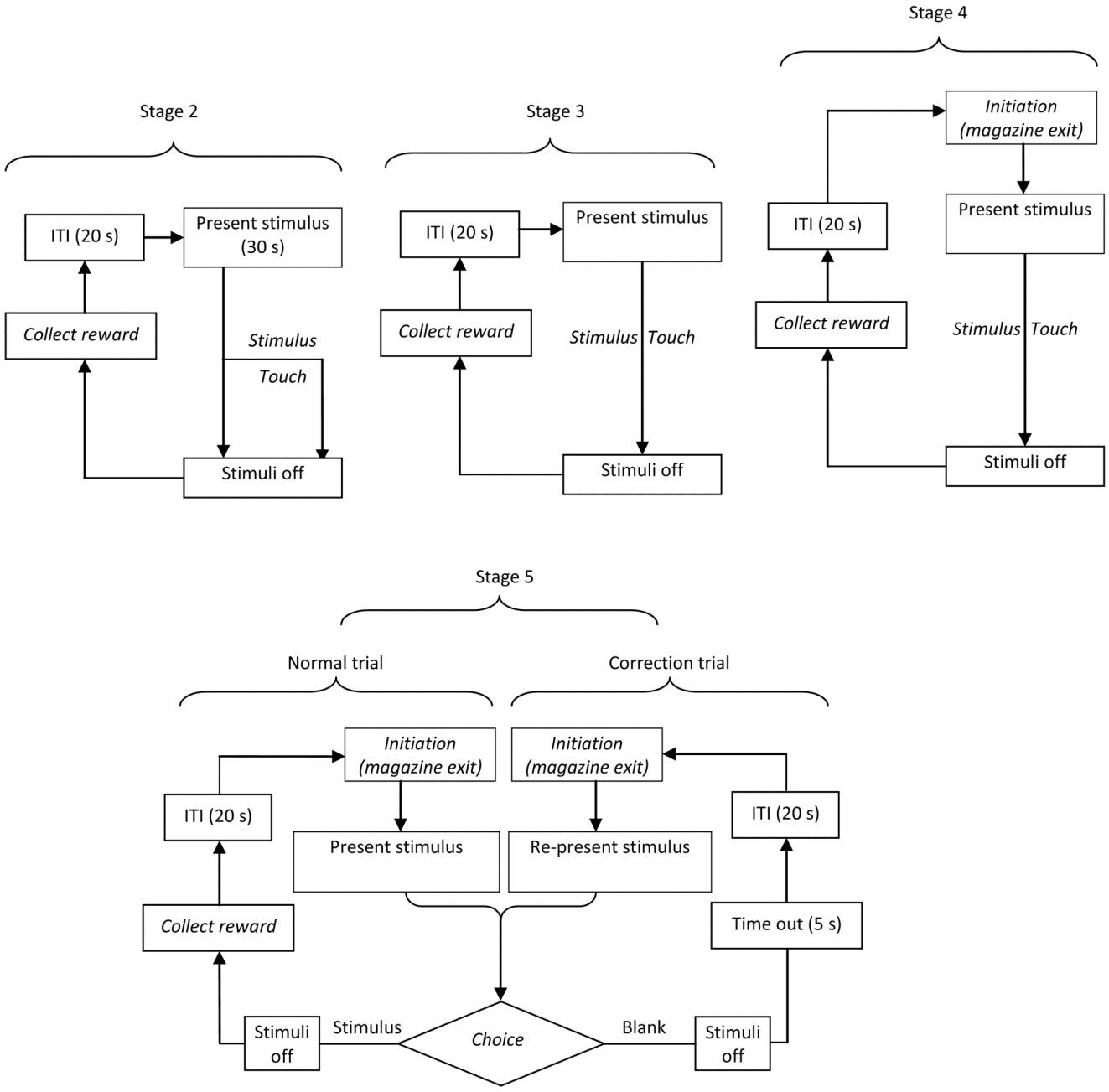


**Figure 1.**

Flowchart overview of pretraining stages 2-5. Stage 2: A visual stimulus is presented in one of the response windows. If not touched, stimulus offset occurs after 30 s and a reward is delivered. If touched, offset is immediate and a triple reward is delivered. After reward collection and an ITI period, the next stimulus is presented in a new trial. Stage 3: Proceeds as in Stage 2, but the stimulus remains on the touchscreen until touched. Stage 4: Proceeds as in Stage 3, but the animal must enter and exit the magazine after the ITI to initiate the next trial. Stage 5: Proceeds as in Stage 4, but touches to blank response windows (when there is a stimulus on the screen) are discouraged with a time out. Following this and the ITI the next trial may be initiated, but in pretraining for the majority of tasks this is a CT in

which the previous stimulus is represented, rather than a new trial. Note that CTs are not given in Stage 5 of pretraining for LD and 5-CSRT. The labels in italics indicate steps in which the animal is required to perform an action.



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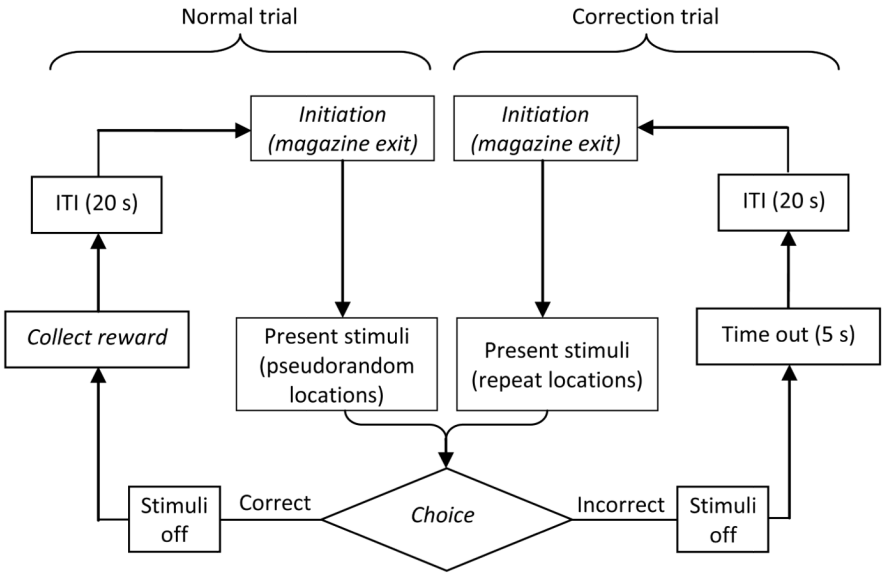


**Figure 2.**

Flowchart overview of Visual Discrimination task. Following initiation, a pair of stimuli (CS+, CS−) is presented on the screen, in pseudorandom locations. Correct responses (to CS +) are rewarded, and after reward collection and an ITI, a new trial may be initiated. Incorrect responses (to CS−) are discouraged with a time out, then after an ITI and initiation the previous trial type is represented (a CT). The CT loop will continue until a correct response is made. The labels in italics indicate steps in which the animal is required to perform an action.

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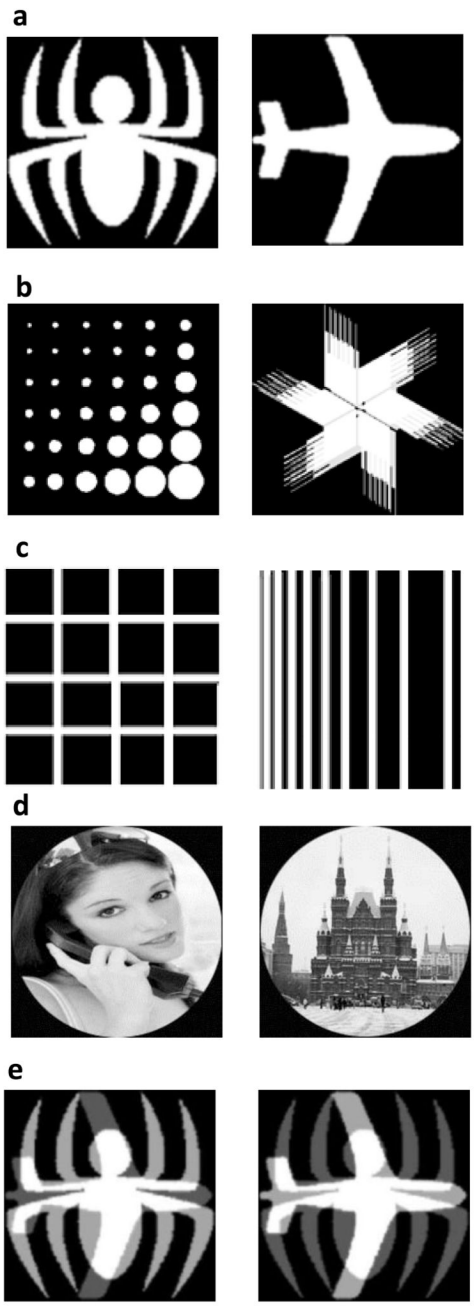


**Figure 3.**

Stimulus pairs recommended for use in Visual Discrimination and Reversal learning. (a) "Spider-Plane" (reproduced from[3] with permission), (b) "Marble-Fan" (reproduced from[63] with permission not required), (c) "Grid-Lines", (d) photographic "Face-Building" (reproduced from[3] with permission), (e) Morphed "Spider-Plane" (60%/40 %; reprinted from Pharmacology, Biochemistry and Behaviour, vol. 98, A. D. McCarthy, I. J. Owens, A. T. Bansal, S. M. McTighe, T. J. Bussey & L. M. Saksida, "FK962 and donepezil act synergistically to improve cognition in rats: Potential as an add-on therapy for Alzheimer's disease", p. 76-80, Copyright 2011, with permission from Elsevier).

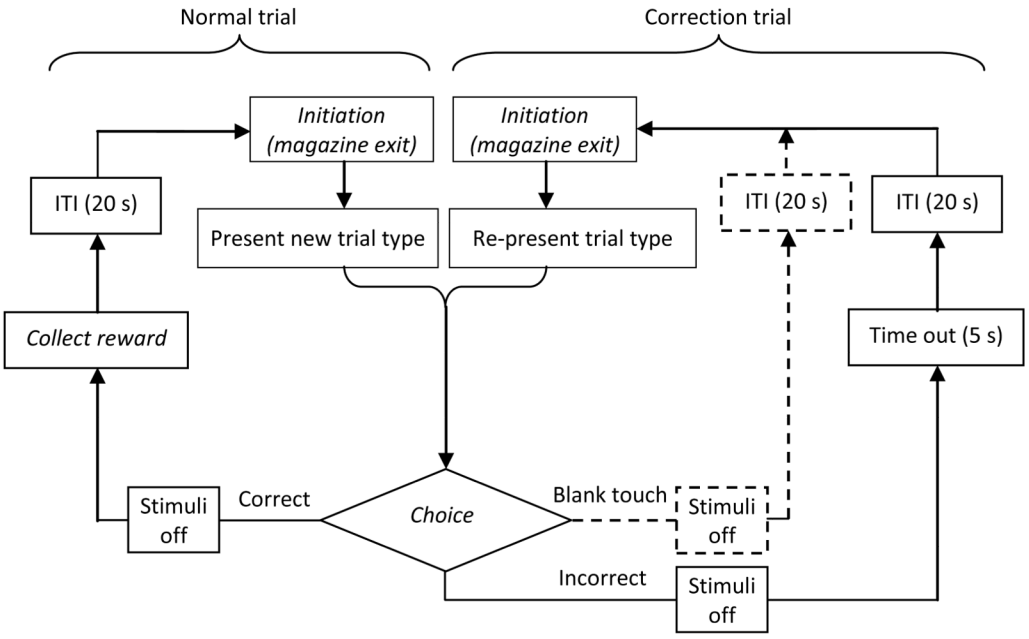


**Figure 4.**

Flowchart overview of Object-Location Paired-Associates Learning task. Following initiation, one of six possible trial types (see Fig. 5), each composed of one CS+ and one CS −, is presented on the screen. Correct responses (to CS+) are rewarded, and after reward collection and an ITI, a new trial may be initiated. Incorrect responses (to CS−) are discouraged with a time out, then after an ITI and initiation the previous trial type is re-presented (a CT). The CT loop will continue until a correct response is made. In our recent task development, we have introduced a consequence for touching the blank location (dashed lines). The labels in italics indicate steps in which the animal is required to perform an action.




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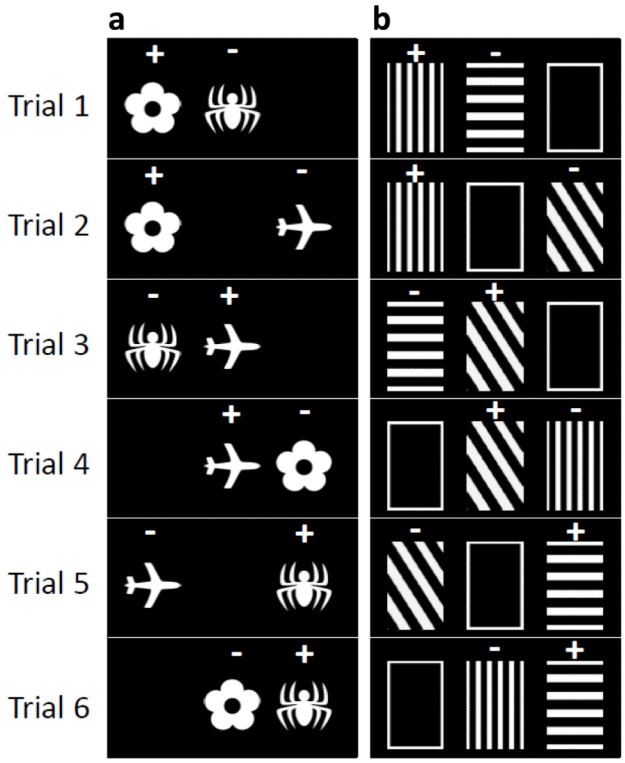


**Figure 5.**

The 6 possible trial types in the Object-Location Paired-Associates Learning task. The stimuli in the left panel (a) are the basis of this protocol and of all published material using the touchscreen Object-Location Paired-Associates Learning task. However, in our recent rat task development, we have used the stimuli in the right panel (b). CS+ (correct choice) is denoted "+". CS− (incorrect choice) is denoted "-". When using standard stimuli (left panel), touches to the blank location are ignored. When using stimuli in the right panel, the blank location is framed white, and touches to it are discouraged (see Fig. 4). Figure (a) is reproduced with kind permission from Springer Science+Business Media: Psychopharmacology, "A novel touchscreen-automated paired-associate learning (PAL) task sensitive to pharmacological manipulation of the hippocampus: a translational rodent model of cognitive impairments in neurodegenerative disease", vol. 205, 2009, p. 157-168, J. C. Talpos, B. D. Winters, R. Dias, L. M. Saksida & T. J. Bussey, Fig. 1, and any original (first) copyright notice displayed with the material.

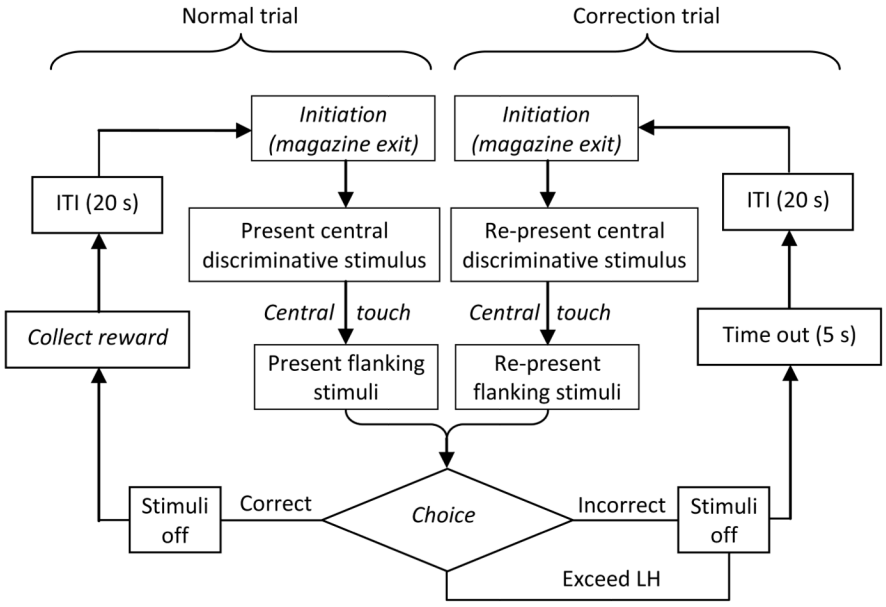


**Figure 6.**

Flowchart overview of Visuomotor Conditional Learning task. Following initiation, one of two discriminative stimuli is presented. Touching this stimulus results in the additional presentation of two choice stimuli in the flanking locations (left and right). The correct/incorrect response choice is determined by the discriminative stimulus, e.g., Stimulus A indicates that right is correct. The subject must respond within the LH period (usually 2 s). Correct responses are rewarded, and after reward collection and an ITI, a new trial may be initiated. Incorrect and absent (LH exceeded) responses are discouraged with a time out, then after an ITI and initiation the previous trial is represented (a CT). The CT loop will continue until a correct response is made. VMCL-specific pretraining trials progress in a similar manner, with some differences. The central stimulus is plain white, rather than discriminative. When it is touched, it is removed and replaced with a single plain white flanking stimulus. Finally, an “incorrect” response is instead defined as touching the blank flanking location. The labels in italics indicate steps in which the animal is required to perform an action.

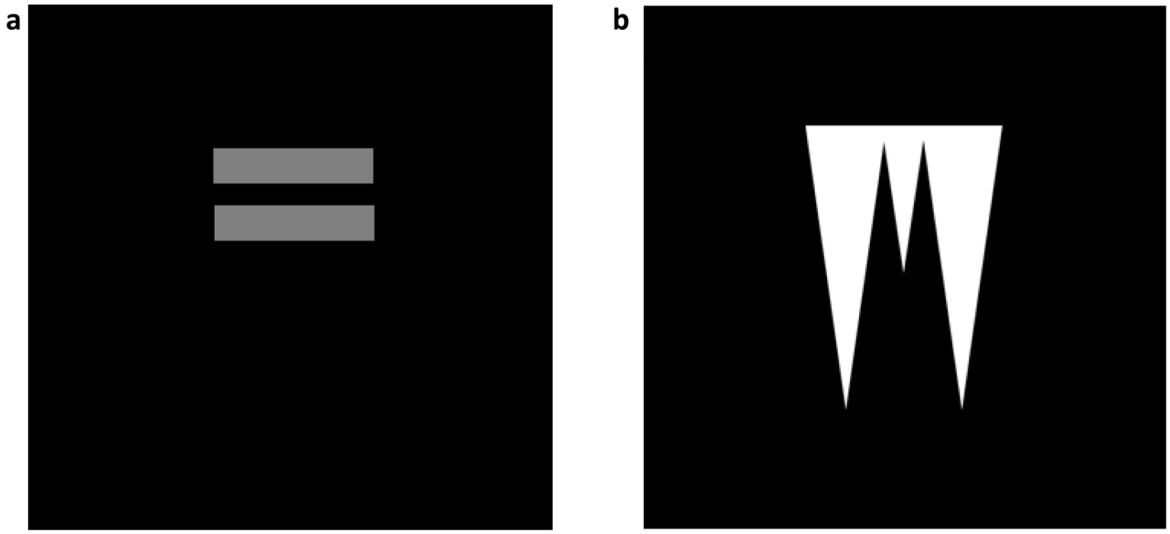


**Figure 7.**
VMCL discriminative stimuli. Reward contingencies are counterbalanced but, for example, Stimulus A (a) may indicate that the right choice stimulus will be correct (and left incorrect), whilst Stimulus B (b) indicates that left will be correct (and right incorrect). Figures courtesy of Campden Instruments Ltd.


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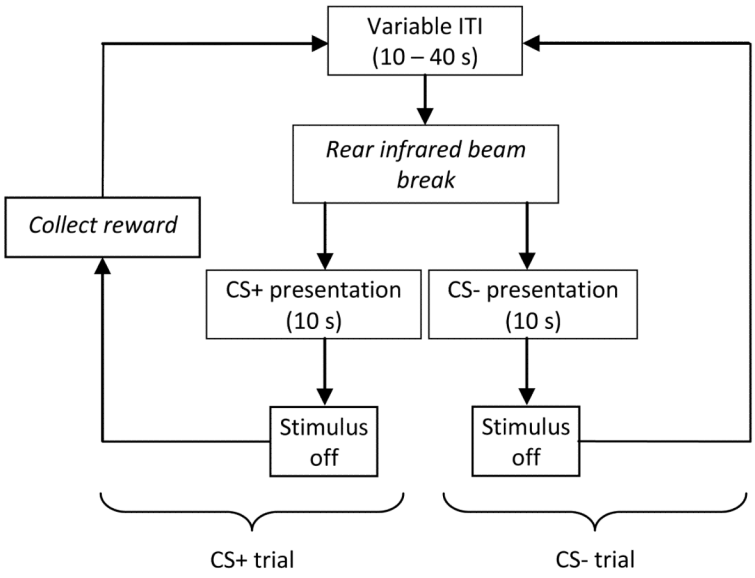


**Figure 8.**

Flowchart overview of the Autoshaping task. Following a variable ITI, a trial is initiated when the animal breaks the infrared beam at the rear of the chamber and a stimulus is displayed (CS+ or CS−). Regardless of the animal's behavior, stimulus offset occurs after a prespecified display time. Upon CS+ offset a reward is delivered, and when the animal enters the magazine to collect it, another variable ITI begins. Upon CS− offset, reward is not delivered and another variable ITI begins. CS+ and CS− trials are organised in pairs, such that if CS+ is presented first, a CS− trial follows. The labels in italics indicate steps in which the animal is required to perform an action.



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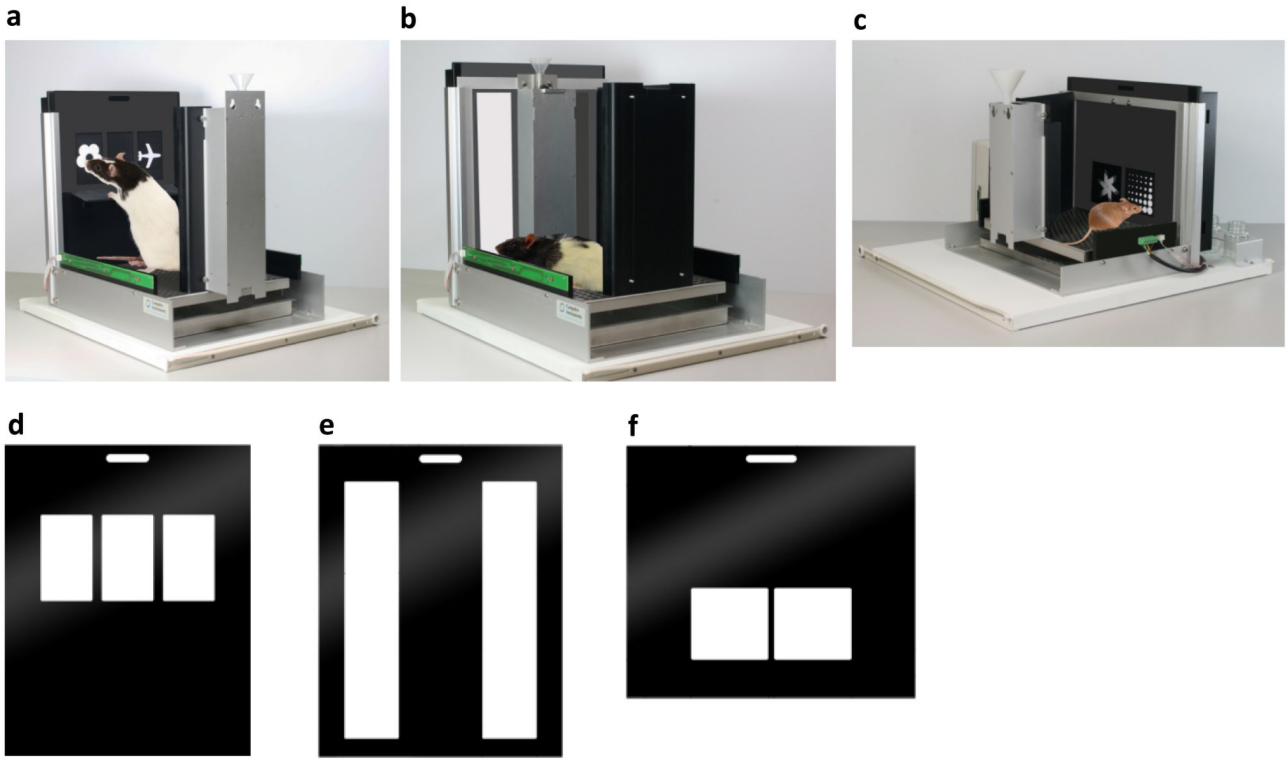


**Figure 9.**
Illustrations of Campden Instruments Ltd. touchscreen chamber apparatus: (a) normal rat chamber configuration, with shelf, showing rat performing Object-Location Paired-Associates Learning; (b) Autoshaping rat chamber configuration, showing rat performing Autoshaping task; (c) normal mouse chamber configuration, showing mouse performing Visual Discrimination; (d-f) black plastic masks which are used to cover the touchscreen in (a-c). Figures courtesy of Campden Instruments Ltd.


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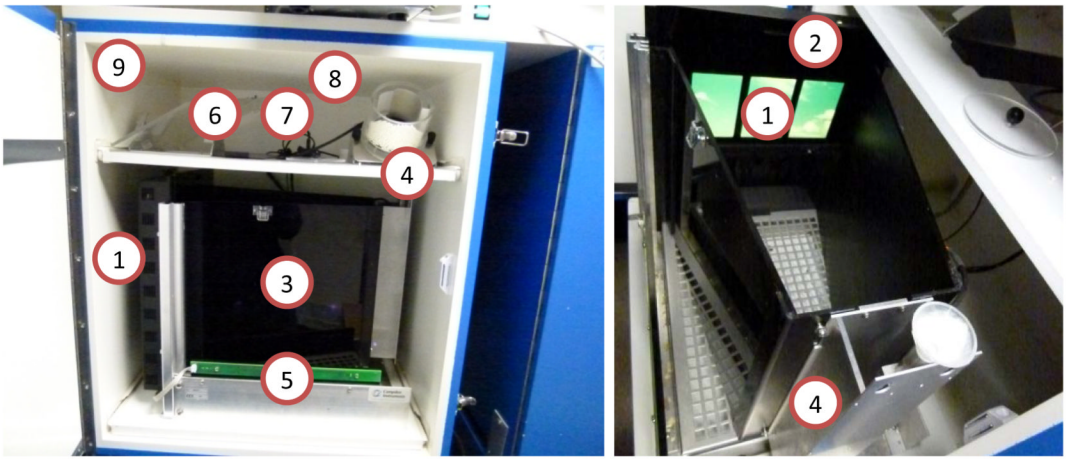


**Figure 10.**
Annotated photographs of a Campden Instruments rat touchscreen chamber. (1) Touchscreen, (2) black plastic mask covering touchscreen except for response windows, (3) black Perspex walls, (4) pellet dispenser (optional), (5) infrared beam assembly, (6) houselight positioned above chamber, (7) infrared camera positioned above chamber, (8) tone and click generator, (9) sound/light-attenuating box with ventilation fan fitted.


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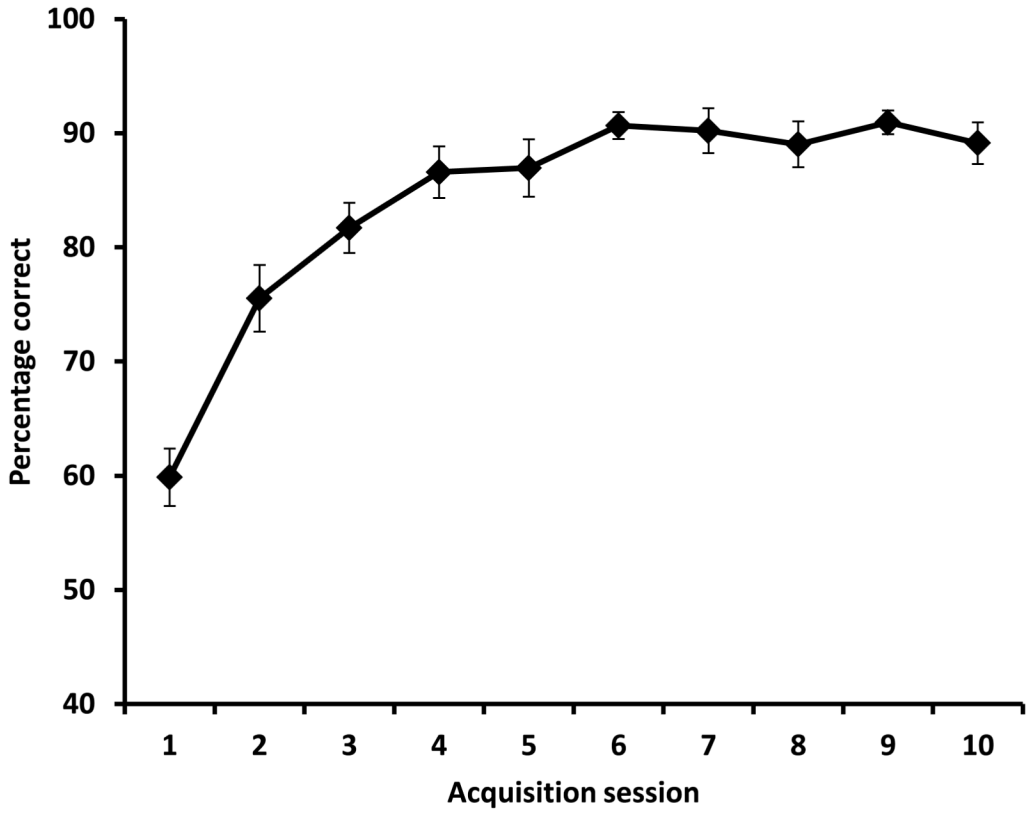


**Figure 11.**
Visual Discrimination acquisition of 10 month old sham-lesioned control rats (n = 10, with a history of PAL and TUNL) using photographic stimuli (C.A.O., unpublished data). Data presented as mean ± SEM.

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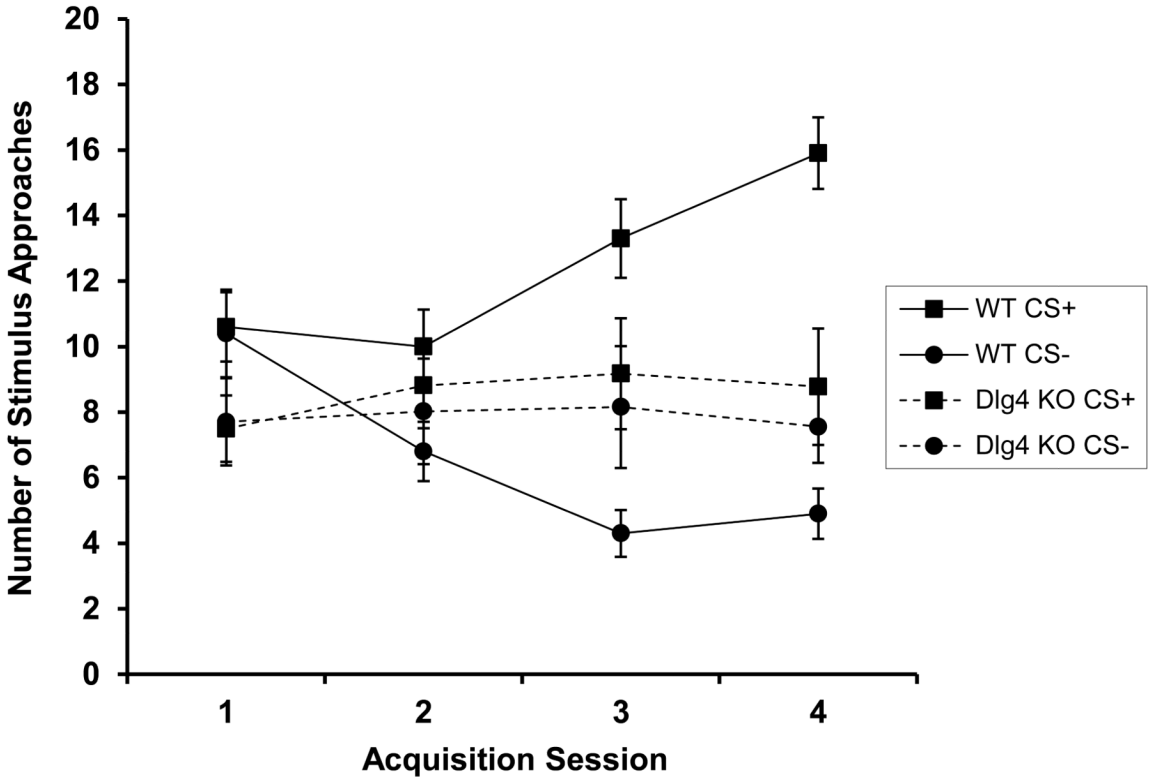


**Figure 12.**

Data from the Autoshaping task. Number of approaches made by WT and Dlg4$^{-/-}$ mice (n = 10-15) to the CS+ and CS− over 4 acquisition sessions[8]. Data presented as mean ± SEM. Adapted by permission from Macmillan Publishers Ltd: Nature Neuroscience[8], copyright 2013.

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## Table 1

Mask and stimulus dimensions. All dimensions are approximate, and given as height × width, in cm. Window gap is the horizontal distance between windows. Floor gap is the vertical distance between the bottom of the stimulus window and the floor. Stimuli are always positioned centrally on the horizontal axis of the screen. Abbreviations/acronyms: Auto – Autoshaping, PAL – Object-Location Paired-Associates Learning, VD – Visual Discrimination,VMCL – Visuomotor Conditional Learning.

| Species/number of windows/type | Task | Window size | Window gaP(s) | Floor gap | Stimulus size |
|---|---|---|---|---|---|
| Rat/2/In-house | VD | 15.0 × 9.2 | 2.5 | 12.5 | 9.0 × 9.0 |
| Rat/2/Campden | VD | 10.0 × 10.0 | 1.0 | 16.0 | 8.5 × 8.5 |
| Mouse/2/In-house | VD | 7.0 × 5.3 | 0.5 | 1.5 | 4.0 × 4.0 |
| Mouse/2/Campden | VD | 7.0 × 7.5 | 0.5 | 1.5 | 5.5 × 5.5 |
| Rat/2/Campden | Auto | 30.0 × 6.4 | 9.6 | 0.0 | 25.8 × 5.7 |
| Mouse/2/Campden | Auto | 17.5 × 8.2 | 7.5 | 0.0 | 15.7 × 7.3 |
| Rat/3/In-house | PAL, VMCL | 15.1 × 6.0 | 1.5 | 12.5 | 6.0 × 5.7 |
| Rat/3/Campden | PAL, VMCL | 10.0 × 6.0 | 1.0 | 16.0 | 6.0 × 6.0* |
| Mouse/3/In-house | PAL | 5.7 × 5.7 | 0.8 | 1.5 | 5.0 × 5.0 |
| Mouse/3/Campden | PAL | 7.1 × 7.1 | 0.4 | 1.5 | 6.0 × 6.0 |

*New rat Paired Associates Learning stimuli (Fig. 5b) are 10.0 × 6.0.

**Table 2**

Problem solving

| Problem | Possible Reason | Solution |
|---|---|---|
| Incomplete consumption of reward | Animal insufficiently food restricted | Decrease weight as regulations permit |
| | Animal insufficiently habituated to reward | Provide reward in home cage for additional days |
| Unstable or poor performance | Low or excessive motivation | Closer attention to weight control; consider temporary feeding separation, according to rate of responding |
| | Aversion to mask or touchscreen | Increase exploration of the mask and screen by applying food reward on the mask (e.g. peanut butter, pellets or other) |
| | Excessive fighting in home cage | Monitor home cage and general health of animal, separate if necessary |
| | Stressors in housing room (e.g. noise) | Make frequent observations of room and cage, move if necessary |
| | Poor learning ability | Exclusion may be necessary |
| Abrupt decline in performance and/or trial completion | Touchscreen error (e.g. non-responsiveness, not displaying images) | Check physical connections, clean, run test program (if available), recalibrate, reboot the system |
| | Reward delivery has ceased or is inconsistent | Check for physical blockage/disconnection, check for interface error, replace |
| | Initiation not detected | Clean magazine photobeam, check physical connections, replace if faulty |
| | | For Autoshaping: clean and test rear infrared beams |
| | Controlling system error (software or hardware) | Check physical connections, reboot the system, change hardware if necessary |
| Animal appears to make unusually low/high number of beam crosses (Campden only) | Infrared beam failure | Clean infrared beam pathway, check position of infrared switch, replace faulty beams |