**Editor's summary:** In this Review, the authors describe how the use of touchscreen-equipped apparatus in behavioral sciences can facilitate the implementation of the 3Rs principles in this discipline and offer a new platform to monitor laboratory animal welfare.

# Using touchscreen-delivered cognitive assessments to address the principles of the 3Rs in behavioural sciences

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**Abstract**

Despite considerable advances in both *in silico* and *in vitro* approaches, *in vivo* studies that involve animal model systems remain necessary in many research disciplines. Neuroscience is one such area, with studies often requiring access to a complete nervous system capable of dynamically selecting between and then executing a full range of cognitive and behavioural outputs in response to a given stimulus or other manipulation. The involvement of animals in research studies is an issue of active public debate and concern and is therefore carefully regulated. Such regulations are based on the principles of the 3Rs of *Replacement*, *Reduction* and *Refinement*. In the sub-specialty of behavioural neuroscience, *Full/Absolute Replacement* remains a major challenge, as the complete *ex vivo* recapitulation of a system as complex and dynamic as the nervous system has yet to be achieved. However, a number of very positive developments have occurred in this area with respect to *Relative Replacement* and to both *Refinement* and *Reduction*. In this Review, we discuss the *Refinement*- and *Reduction*-related benefits yielded by the introduction of touchscreen-based behavioural assessment apparatus. We also discuss how data generated by a specific panel of behavioural tasks developed for this platform might substantially enhance monitoring of laboratory animal welfare and provide robust, quantitative comparisons of husbandry techniques to define and ensure maintenance of best practice.

## Introduction

Given the continuing public debate and concern related to the involvement of animals in biomedical research, the regulatory environment for this type of research can be very stringent. The implementation of such regulations requires careful consideration of the merit and necessity of any proposed research against implications for the welfare of the animals involved[1]. As part of this consideration, investigators are often required to offer a comprehensive justification for the proposed animal use, demonstrate an active consideration of any potential non-animal alternatives and generate a detailed experimental design indicating how many animals would be required, how they would be manipulated and the risks to the animals associated with those manipulations[2]. Investigators should also ensure that the proposed number of animals is adequately justified and that all possible measures to mitigate any risks to animal welfare have been implemented in the given experimental context.

Consequently, the 3Rs Principles of *Replacement*, *Refinement* and *Reduction* (see Table 1 for the definitions originally promulgated in 1959[3]), are an integral part of key regulatory frameworks such as the UK Animals (Scientific Procedures) Act 1986 as amended by EU Directive 2010/63/EU[4] and the US Guide to the Care and Use of Laboratory Animals[5]. The importance of these principles has also led to the establishment of a number of 3Rs-focused agencies and organisations including the UK National Centre for the 3Rs (NC3Rs), the Centro 3R in Italy, Norway's Norecopa[6], the Canadian Council on Animal Care (CCAC)[7], the German Centre for the Protection of Laboratory Animals (Bf3R)[8] and Charité 3R[9] to actively promote, support and provide guidance on the integration of these concepts into research practice.

In behavioural neuroscience, *Replacement*[3,10–12] is particularly challenging as this discipline typically requires the study of a complete nervous system that can access, select and execute a full range of behavioural and cognitive outputs in response to a given stimulus or manipulation. Indeed, despite major advances in both *in vitro* approaches and *in silico* neural modelling, complete *ex vivo* recapitulation of such a dynamic system in this research area − so-called *Full or Absolute Replacement*[10] of animals − remains to be realised. However, additional granularity can be applied to the definition of *Replacement*, through the use of the terms *Partial Replacement and Relative Replacement*[3,10–12]. These terms refer to approaches involving organisms that are not currently considered to be able to experience suffering[11,13]. Alternatively, as specified in the ILAR Guidelines for the Care and Use of Laboratory Animals in Neuroscience and Behavioural Research, these terms refer to approaches "replacing animals such as vertebrates with animals that are lower on the phylogenetic scale"[12,14].

In practice, this is often viewed as the replacement of vertebrate systems with invertebrates and in neuroscience, the nematode *Caenorhabditis elegans*[15], the fly *Drosophila melanogaster*[16] and some sea urchin species[17] have shown considerable promise for certain types of study. However, making 'smaller' steps down the phylogenetic tree is also a valuable approach for *Replacement*. For example, rodents may serve as an acceptable substitute in some studies that would have traditionally involved non-human primates (NHPs)[18,19].

Consequently, while progress is being made with respect to *Replacement,* the majority of behavioural neuroscience research still requires the use of vertebrate animals and much attention is currently focused on the implementation of *Reduction* and *Refinement* in this discipline[2,20–22].

Since its initial development in the 1990s[23–25], touchscreen-equipped behavioural assessment equipment has become increasingly widespread in behavioural neuroscience and is now available for a range of species, including mice, rats and several primate species[26–30] (box 1). While there are variations in design dependent on manufacturer and species, most touchscreen-equipped apparatus is broadly similar with respect to features, capabilities and their 3Rs-related benefits, in particular *Refinement* and *Reduction*-related benefits.

In the present Review, we will discuss the application of the 3R in behavioural neuroscience, and highlight how developments in operant behavioural assessment, and in particular the development of touchscreen-equipped apparatus can further facilitate the implementation of the 3Rs principles in this discipline. We will also describe how this equipment may provide a new, standardised approach to animal welfare monitoring and determination of best practice .

## 3R considerations in behavioural neuroscience

The application of the 3Rs principles to behavioural neuroscience research activity begins at study design. The objective of many studies in this discipline is to elucidate the neurobiological basis of a specific cognitive construct or behavioural process. Given this objective, investigators must first establish an appropriate balance between the degree of sentience exhibited by the different model organisms (i.e. NHPs vs. rodents vs. zebrafish vs. invertebrates) that could be selected for the study and the capabilities of each model to express the behaviour or cognitive construct of interest, with a view towards maximised *Relative Replacement*. In the disease context, the selected animal model (and its behavioural,

cognitive or pathological phenotype) ideally must also mimic as closely as possible the pathogenesis, progression and symptomology of the illness under study ('Face validity'[31]).

After selecting the model species, investigators must determine an approach to measure the behaviour or construct of interest appropriate to that species[32]. Validity is an important factor, in that the selected assay should be robustly validated with respect to the construct of interest ('construct validity'[31]) and assay performance should respond predictably to a given manipulation ('predictive validity'[31]). To maximise the probability that behavioural and cognitive insights derived from model systems can be applied to humans and particularly the clinical context, assays should also ideally mirror approaches used in humans as closely as possible ('translational validity'[31,33]).

Where behavioural neuroscience studies incorporate behavioural pharmacology, for example to determine the effect of a new compound on the construct of interest, investigators must take into account additional study design considerations. These include possible species-dependent effects on the route or rate of compound metabolism, on the expression patterns or biophysical characteristics of the molecular target(s) of the compound and on differential off-target activities. The administration route, the vehicle within which the compound can be delivered and the potential adverse effects must also be considered.

Investigators should also consider if the intended approach can undergo any *Refinement*. This can be achieved by ensuring where possible the selection of behavioural tasks or assays that are free of aversive stimuli or stressful conditions such as water immersion, restraint or inescapable footshock and the use of compound administration routes such as via cage drinking water supplies, treated food or non-gavage oral administration with a highly palatable vehicle[34]. *Refinemen*t of other aspects related to the life of the animals including husbandry techniques must also be considered[35,36].

Beyond selection of the animal model, behavioural assay, and approach to exogenously manipulate the nervous system, experimental design and statistical model selection are also critical for the application of the 3R, as these factors can impact the number of animals required (potential for *Reduction*). For example, the use of within-subject or longitudinal designs can substantially reduce animal numbers relative to the between-subject or cross-sectional alternatives and will reduce variability[37,38]. An increasing number of online resources that can inform and support such determinations (for example[39]) are available. Furthermore, with respect to *Reduction*, the per-animal data yield generated by a particular assay must also be considered. Assays that simultaneously generate multiple outcome

measures beyond the 'primary' performance metric (e.g. percentage of trials correct or time spent exploring a location) are to be favoured. These assays may eliminate the need for the same cohort to undergo further study or for an independent cohort to be evaluated with a different assay. More broadly, *Reduction* can also be facilitated by increased standardisation of methods both within and between laboratories that perform research in similar areas of behavioural neuroscience, thereby facilitating comparison and aggregation of data, enhancing replicability and decreasing needless duplication of studies using similar but non-identical techniques[40].

Taken together, it is clear that the 3Rs principles have important implications for behavioural neuroscience study design. In the next sections, we will describe how the use of touchscreen-equipped apparatus in behavioural sciences can further facilitate the implementation of the 3Rs principles in this discipline.

## Touchscreen-equipped behavioural apparatus and the 3Rs

**Translational potential.** Current estimates indicate that >90% of clinical trials in neuroscience end in failure. Several potential factors have been identified[41], but evidence suggests that discrepancies between how psychological functions are assessed in experimental animals and humans greatly contribute to this problem. Therefore, behavioural neuroscience studies evaluating the efficacy of a novel manipulation or putative therapeutic intervention should be designed to yield outputs that can be more effectively translated into the clinical context.

The advent of touchscreen-equipped behavioural equipment for laboratory species has enabled the adaptation and in some cases, the direct translation, of computerised cognitive assessments used in humans such as the Cambridge Neuropsychological Test Automated Battery *(*CANTAB)[42–44] and the EMOTICOM battery[45] to key vertebrate model systems. This development has permitted the assessment of cognitive domains such as learning, memory and executive function as well as various aspects of motivation and affective state in laboratory species[46] using an approach that closely mirrors the approach used to evaluate the same constructs in clinical populations[26,47,48]

The cross-species translational potential of the touchscreen system can be evidenced by the similarities in paired-associates learning (PAL) task performance observed between mice and humans expressing mutations in the neuropsychiatric disease-related *Dlg2* gene[26,49]. Similarly, parallels can be drawn between the performance of the touchscreen PAL task and other touchscreen-based tasks assessing attentional and executive function in

patients with Alzheimer's disease and a mouse model of the disease[50–52], and when motivation is evaluated in patients with Huntington's disease and the R6/1 mouse model of this disease[53]. The human probabilistic reward task (PRT) was adapted for use in 2020 in the rat touchscreen system, which demonstrated similar performance outcomes to those observed in humans[54].

Further evidence of the translational potential of the touchscreen platform can be derived from behavioural pharmacology studies, when compounds with known effects in humans performing a particular cognitive task have similar effects in other species performing the same task. As an example, the touchscreen Continuous Performance Task (CPT), which was originally designed to assess executive control and sustained and selective attention in humans has since been adapted for both rodents[55] and NHPs[56]. The touchscreen CPT assay takes advantage of the unique capacity of the touchscreen system to display more complex and varied stimuli than would be possible in a standard animal operant chamber[57] to more accurately recapitulate the human paradigm. A number of recent studies in which mice and rats were administered a panel of compounds used clinically for the treatment of ADHD revealed effects in the touchscreen CPT congruent with those reported in humans[58–60]. An earlier study in which both humans and mice performed a related task called the 5-choice-CPT (5C-CPT) also revealed analogous effects of administration of the psychostimulant D-amphetamine[61]. The performance in the 5C-CPT also seems to be similarly impacted in mice and humans perinatally exposed to alcohol[62]. Finally, the adaptation of the Iowa gambling task in the mouse touchscreen system has indicated that the effects of the administration of psychedelic compounds on performance are similar to those observed in humans using the same paradigm[63].

These validation studies demonstrate the high translational potential of data derived from touchscreen behavioral assessment in laboratory rodents, which could potentially improve therapeutic discovery success rates. Ultimately this improvement could also result in fewer animals being involved in therapeutic screening programs if data derived from preclinical *in vivo* studies more reliably predict the clinical efficacy (or lack thereof) of putative therapeutics.

**Relative Replacement.** The rapid development of assays for rodent touchscreen assessment apparatus[48,64–66] has revealed the considerable capabilities of these species. In some research areas, the introduction of this technology could facilitate *Relative Replacement*, as certain studies that would have previously been performed in NHPs on the basis of their higher phylogenetic position could be performed instead, or at least initially, in rodents.

As an example, the availability of touchscreen assessment systems for rodents has coincided with the increasing recognition of the inherent sophistication of the rodent visual system, with recent research indicating evidence of characteristics previously considered exclusive to NHPs[67–69]. Given that the touchscreen system is also highly versatile with respect to the characteristics of the visual stimuli an animal can interrogate (e.g. stimuli size, shape, color and location can be easily modified and adapted), a number of studies have been performed to establish important baseline characteristics of the rodent visual system in the touchscreen apparatus[70–72]. Such studies have identified key similarities and differences between the rodent and human visual systems, which are critical to the design and interpretation of studies using the touchscreen apparatus to challenge various aspects of the rodent visual system[73–76]. Consequently, the integration of the rodent model in robustly validated touchscreen tasks designed to target higher order visual cognition may facilitate *Relative Replacement* of NHPs in vision research.

**Refinement.** A key aspect of good practice in behavioural assessments is ensuring consistency in the stimuli presented in the assay as well as in the wider environment in which the assay is being performed. Consistency is important to minimise variability, maximise replicability in the data produced and to ensure that animals are not inadvertently exposed to unexpected aversive environmental stimuli which may cause distress. Consequently, in behavioural neuroscience, all efforts are made to control stimuli and the environment, irrespective of the species, assay or apparatus being used. In common with non-touchscreen operant assessment apparatus, touchscreen systems contribute to these efforts by providing a greater degree of environmental control relative to open field or maze-based assessments. Specifically, by enclosing the behavioural arena in a sound-attenuating chamber, animals are better insulated from external stimuli and consistently experience a standardised cue environment. This setup makes the animal less vulnerable to non-task related stimuli and potential distractors (e.g. unexpected loud noise, different intensities of light[77]) which may be present inconsistently during and between behavioural assessment sessions.

In addition to this feature and others common to most operant apparatus (e.g., animals are typically habituated to the operant chamber for the duration of assessment to avoid neophobia; operant chambers are typically small enough to minimise anxiogenesis associated with the need to traverse a central open space and the expression of thigmotaxis; operant chambers can be used to support performance and monitoring of behaviours in darkness or low light conditions which are favoured by nocturnal rodent species), the touchscreen apparatus offers other unique *refinement* opportunities.

As an example, touchscreens offer a far greater number of unique spatial locations in which stimuli can be presented within trials and greater temporal control over stimulus presentation compared with conventional operant apparatus. Touchscreens also provide the opportunity to make changes to other stimulus properties (e.g. size, shape and signal-to-noise ratio) while a behavioural session is underway (i.e. without having to physically add or change the position or settings of stimulus light modules within the chamber which typically requires partial disassembly).

This flexibility has facilitated the development of a range of touchscreen tasks to evaluate spatial memory, spatial working memory and pattern separation[78]. These assays avoid the need to expose animals to a large open environment − a setting that facilitates exploration and ensure adequate separation of cues − or to place them in a water tank such as in the Morris Water Maze (MWM) in which animals swim to find a platform and memorise its location, and in which the stressor is inescapable during 'probe trials'[79–82]. The MWM increases animal corticosterone levels, an accepted proxy measure for stress[83]. In addition to representing a welfare concern, the stress induced by this task can interfere with the interpretation of results and animal performance[83,84]. Two independent studies compared the performance of two Alzheimer's mouse models (APP-KI and APP/PS1) on the MWM to the performance of touchscreen PAL, location discrimination (LD) and visual discrimination and reversal tasks; these studies reported that the touchscreen tasks detected significant performance impairments in the model animals compared to wild-type controls at an earlier age (and so under conditions of lesser pathological burden) than the MWM[52,85]. These findings suggest that the touchscreen approach can offer higher sensitivity assessment with lower stressor exposure, consistent with a substantial *refinement*.

*Refinement* can also be applied to the valence of the stimuli delivered in a behavioural assay. While it is important to acknowledge that in some research areas, the delivery of negatively valenced stimuli that can cause pain or distress is accepted as standard practice and can be necessary for studying psychological processes (e.g. foot shocks in fear conditioning), as is the exposure of animals to stress-inducing contexts (e.g. social isolation, restraint, cold/wet caging conditions); to date such stimuli and contexts have been avoided with the touchscreen apparatus. All touchscreen tasks characterised so far utilise positive reinforcement in the form of sweetened liquids like strawberry milkshake or solid reward pellets and only require animals to be mildly food restricted to ensure stable and sustainable performance[86]. Such levels of food restriction in rodents are suggested to better model the

human condition[87] and have a variety of physiological benefits[88,89] including an increase in maximum lifespan[90] by preventing or delaying the development of various diseases[91].

In addition, while not necessarily a direct substitute for cued fear conditioning or fear potentiated startle, the touchscreen autoshaping task[23,30] (Pavlovian conditioned approach) has been validated for use as an assay of Pavlovian processes dependent on limbic regions such as the amygdala[92,93] without the need for footshock administration. A recently characterized touchscreen cognitive judgement bias task also avoids footshock administration. Tasks evaluating affective state bias in decision making often involve the administration of footshocks to represent negative emotional valance[94,95], however, the touchscreen variant instead uses a brief time-out period under inverted illumination conditions to achieve the same effect[96,97].

While not necessarily unique to the touchscreen apparatus, the development of non-aversive touchscreen assays targeting constructs commonly accessed in non-touchscreen paradigms through application of aversive stimuli can be taken as an indicator of the direction of travel that wider implementation of this equipment will have in behavioural neuroscience.

**Reduction.** Touchscreen-equipped behavioural assessment chambers can contribute to *Reduction* by decreasing animal numbers, increasing per-animal data-yields and facilitating the sharing of data and resources between investigators.

***Decreasing animal numbers and increasing per-animal data-yields.*** Decreasing the overall number of animals involved in a study and maximising the amount of data generated per animal are two important approaches for *Reduction.* Touchscreen-equipped behavioural assessment chambers can contribute to these aspects of *Reduction*.

Firstly, compared to a number of maze-based and other 'hand run' behavioural assessments, the computerised data collection and analysis system incorporated into touchscreen equipment substantially increases the number of variables that are recorded beyond the typical measures of task performance. For example, in a touchscreen-delivered attentional task like the 5-Choice Serial Reaction Time Test (5-CSRTT), in which the 'primary' aim is usually to evaluate attention or distractibility[98], it is possible to evaluate additional 'secondary' measures that provide information on other related psychological processes such as response inhibition, impulsivity, perseveration and processing speed[98]. Thus a single test

like the 5-CSRTT can be used to simultaneously study multiple cognitive abilities, thereby reducing the number of animals required.

Coupling such variable-rich data streams with the considerable capacity for within-session spatial variation of stimulus presentation offered by the touchscreens has allowed the development of spatially-driven, hippocampus-focused tasks such as the Trial Unique Non-match to Location (TUNL) task[99], the continuous TUNL[100] task and the LD task[101]. These developments represent substantial advances over analogous non-touchscreen operant methods such as the Delayed Non-match to Position (DNMTP)[102] task and other maze-based equivalents, in that they minimise the expression of so-called motoric mediating behaviours that animals can use to bridge delay periods in the tasks, and in doing so confound the data produced[99]. This advantage depends on the ability to use multiple locations on the screen rather than a small and limited number of levers, as is typical in the DNMTP. The touchscreen tasks also facilitate the assessment of pattern separation[79], a process dependent on the hippocampal dentate gyrus which is much more challenging to evaluate in the non-touchscreen operant DNMTP context due to the fixed response manipulanda locations in this equipment.

The availability of such variable-rich data profiles for individual animals may eliminate the need for an investigator to perform additional experiments with further independent cohorts to assess any 'secondary' measures of interest in the context of a given manipulation. In this vein, it is common practice to conduct control experiments to evaluate animal mobility or to detect possible locomotor impairments or hyperactivity derived from a manipulation of interest (e.g. pharmacological, surgical or genetic) and to ensure that any behavioural/cognitive task data are not confounded. For example, in some studies evaluating therapeutic targets for depression using the forced swim test (FST), off-target effects of manipulations on locomotor activity often need to be evaluated in a cohort of animals distinct from that used to screen the manipulation for affective state efficacy[103,104]. The touchscreen-based approach eliminates the need for such experiments and additional animals due to the integration of infra-red activity beams across the behavioural arena which can be used to evaluate locomotion similarly to a standard activity-monitoring cage. Additional measures such as off-target screen touches, magazine entries and latencies (e.g., to collect reward) provide further proxies of locomotion during assessment sessions without the requirement for additional testing in independent cohorts.

Secondly, increasing evidence from the touchscreen literature is showing that it is possible to use within-subject designs and assess single cohorts of rodents across multiple

touchscreen tasks for evaluation of distinct cognitive and behavioural constructs with minimal evidence of task cross-over effects[105–108]. This feature offers a number of benefits, including reductions in the number of cohorts required to fully characterise the effect of a manipulation, assessment within the same environment and using the same type of operant response, thereby reducing training time and decreasing data variability. Within-subject design also enhances confidence in the robustness of a particular phenotype observed in different assays[109], the potential for within-subject correlations between tasks, the capacity to use the findings from one task as rational, data-driven predictors for subsequent assays and the potential to dynamically adjust the package of tasks applied to explore unexpected findings as they emerge from a cohort.

Thirdly, in common with non-touchscreen operant assays, the touchscreen assessment approach can support extended longitudinal behavioural evaluation across multiple tasks in a cohort. This is of particular value to assess the effects of progressive or degenerative manipulations[30,110–112].

***Protocol standardisation and replicability*.** The facilitation of the sharing of data and resources between investigators can also contribute to *Reduction*, as it may prevent needless replication of studies already completed by others[13] and encourage standardisation of protocols. The latter is important in that it enables the direct comparison of findings from different groups using the same assay, which may help to address the perennial concern of replicability[113].

Protocol standardisation is often a major challenge as many factors in the lab environment can have adverse or destabilising effects[114]. A given protocol should not only be performed consistently within a given study and within a given laboratory, but also by all researchers in a given field to enable data comparison and to rule out the possibility that any discrepant results are biologically significant and not a result of procedural differences.

Rodent touchscreen apparatus enhances opportunities for protocol standardisation relative to open maze-based tasks and other ‘hand run’ assays, which can be more susceptible to protocol variation as the apparatus involved is often built to in-house specifications, makes use of laboratory specific cues, contexts and stimuli and is performed based on a protocol optimised exclusively within the laboratory.

In common with non-touchscreen equipped operant apparatus, the touchscreen system can minimise potential discrepancies through the sharing of the programs used for

behavioural assays, including the files containing the visual stimuli displayed on the touchscreen and the programs that generate the performance outcomes from the raw data collected by the apparatus. Importantly, given that touchscreen tasks generally only use the input and output devices in the standard chambers and are primarily driven by the stimuli and responses on the touchscreen, most existing tasks can be shared between any touchscreen-equipped laboratory without the need to purchase additional hardware (e.g., extra lever or stimulus light modules)[115,116]. This potential has led to the development of an international rodent touchscreen knowledge-sharing website, a data repository, a data sharing platform and an Open Science community of practice among researchers using the equipment[116,117]. The touchscreen knowledge-sharing website (https://touchscreencognition.org) contributes to replicability and reproducibility by sharing protocols and promoting communication between researchers. The online data repository (https://www.mousebytes.ca/home) contributes to reduction by allowing data sharing (including negative data), meta-analysis and reuse of data, which reduce the need for running new experiments[117].

In addition, while developments towards increased automation in the performance, particularly the scoring of behaviours, generated in maze-based and other 'hand run' tasks are underway[118–121], operant apparatus such as the touchscreen system continue to offer markedly lower levels of animal–experimenter interaction. Animal–experimenter interactions can potentially have substantial effects on behavioural data due to trial-by-trial or session-by-session variability, for example due to handling differences, or changes in the appearance, smell or physical position of the experimenter over time[122]. The lack of human observer-based scoring of behaviour in operant apparatus also increases consistency by preventing scorer bias and eliminating concerns regarding intra- and inter-rater reliability, which decreases variability between animals, behavioural sessions and laboratories.

The capacity of the touchscreen system to ensure standardisation was demonstrated in a study that compared the performance of mice in the touchscreen 5-CSRTT across laboratories in the UK and South Korea[123]. Even though experiments were performed by independent research groups at different institutions, the performance of animals trained using the same touchscreen protocol, stimuli and analysed with the same data analysis program was statistically equivalent[123]. Laboratories in South Korea and Australia have also reported consistent results when independently evaluating the *mGluR5* knock-out mouse strain across the touchscreen visual discrimination, reversal learning and extinction tasks targeting visual perception, learning and cognitive flexibility/perseveration[124,125].

Taken together, the various aspects of the touchscreen hardware, combined with the high inherent and unique measurement accuracy (in milliseconds) and elimination of subjective bias through automation to maximise replicability, in conjunction with the robust protocol standardisation offered by this approach may enhance the probability of detecting genuine effects, reduce variability within and between laboratories[126] and ultimately decrease the overall number of animals required to determine the effect of a given manipulation.

## Exemplar applications of touchscreen techniques

**Applications for affective state research.** The recent development of a series of touchscreen versions of tasks targeting constructs related to mood and emotional state[28,48,96,127,128] has expanded the possible applications of the touchscreen system to new areas of behavioural neuroscience research by providing a powerful complement to the existing cognition-focused assays.

Examples of these affective state-related construct-focused tasks include the touchscreen implementations of the progressive ratio (PR) schedule for the evaluation of motivation[129,130], the probabilistic reversal learning (PRL) task for the evaluation of negative feedback sensitivity[127,131,132] the affective/cognitive bias task[96,97,128] and the PRT[54]. The development of these tasks is particularly important from a 3Rs perspective because the assessment of mood-related constructs in experimental animals often requires stress induction[30,32], including in widely used assays such as the tail suspension test (TST) and the FST[133].

In both the TST and FST, animals are placed in unescapable situations and the output of the tasks consists of determining the point at which behavioural despair is achieved[134,135]. The FST and TST have both been used extensively to screen compounds for antidepressant efficacy and are canonically considered to have “good predictive validity”[134,135]. However, recent reports suggest that these tests[136] do not mimic human aetiology or behavioural manifestations of depression, raising concerns about their translational potential[137–139] and encouraging funders not to support project proposals using the FST or similar assays[137]. Moreover, similar to the MWM, the stress induced by these assays may have carry-over effects thereby confounding the results of any assay in which TST and/or FST-exposed animals are subsequently involved. More importantly, these assays have persistent negative effects on animal welfare[140,141]. In addition, given the increased sensitivity of touchscreen

tasks relative to the MWM[52,85] , in which animals are also exposed to stress, touchscreen tasks might also show enhanced sensitivity compared to FST and TST, although to our knowledge this important comparison remains to be completed.

Opportunities for refinement of stress-dependent behavioural assays such as the FST and TST should be explored in affective state research. Touchscreen-based behavioural assessments may provide an ideal platform for the further integration of 3Rs practice in this research area.

**Applications for 'home cage' cognitive and behavioural assessment.** Continuing developments in behavioural research methodology have led to the presentation of a touchscreen-based 'home cage' testing approach to completely remove animal–experimenter interactions[142]. This approach consists of an automated touchscreen-based system connected to a rodent home cage via a system of tunnels with computerised access gates that allow animals to individually access the operant behavioural chamber, complete the behavioural session and return to the home cage without any contact with an experimenter[142].

Rats tested in their home cage learned faster than animals manually placed into operant behavioural chambers on a daily basis, and the data produced was highly comparable to results obtained in other laboratories[142]. This approach reduces the potential contribution of animal–experimenter interactions to data variability, as well as the stress associated with the transfers of the animals between home cage and testing environment, or neophobia associated with the testing environment.

Touchscreen-based 'home-cage' testing has also been applied to larger animals including NHPs for both research purposes and as a source of enrichment in non-research contexts[143,144]. For example, in certain studies NHPs can freely access assessment areas from their home environment, and engage with automated touchscreen-based systems to enable cognitive evaluation[144–146]. While representing an advance over conventional methods that necessitate the use of primate chairs, limitations include the need to identify the NHP engaging with the touchscreen system at a given time, which typically requires the introduction of radio-frequency identification implants[144–146]. While not typically considered a major procedure, use of such implants is not without concern and must be considered carefully from a 3Rs perspective[147,148]. A recently developed wireless touchscreen system that utilizes real-time NHP facial recognition to initiate subject-specific tasks, without the need for implanted microchips, shows considerable promise[149].

As such, the ‘home cage’ approach to touchscreen testing[143,150] may ultimately provide additional welfare- and scientific-related benefits across a wide range of species.

## Touchscreens as animal welfare evaluation tools

Regular welfare assessments are an essential component of the husbandry and maintenance of laboratory animals. Aside from monitoring animals for signs of pain or distress during or following an experimental procedure, welfare assessment also encompasses the routine evaluation of animals to check for any health or welfare-related issues in the home cage. These assessments are usually performed through visual inspection of a range of behaviours and physical characteristics that can indicate issues such as injury, dehydration or infection/illness[151,152]. While very effective for the detection of major welfare concerns, such an approach does have limitations and might not detect changes in affective state and/or motivation that still severely impact quality of life.

Welfare assessments of this type typically yield qualitative data, which, while useful, is also limited with respect to comparability across housing rooms, facilities or institutions. This is a critical factor, as considerable variation can be observed in standard procedures across facilities, including caging type (e.g. conventional vs. individual ventilated cages), room illumination, room population density, cage population density, ambient sound levels, cage change procedures and environmental enrichment provision. While all facilities do the utmost to maximise animal welfare, determining best practice in the absence of quantitative measures is challenging.

Quantitative scales such as the grimace scale can provide insight into welfare[153,154], but evidence of considerable baseline variation[155] and dependence on a human observer/scorer limit their utility for routine cage-side welfare assessment.

Given that it provides a number of benefits such as elimination of scorer bias, variable-rich quantitative data streams, non-invasive and non-aversive assessments that facilitate longitudinal evaluations and a suite of tasks known to be sensitive to changes in several affective state-related constructs, the touchscreen system may represent an ideal platform upon which to base welfare assessments. In addition, the literature now contains a number of examples of studies in which animals with known degenerative phenotypes are able to engage effectively with the apparatus at elevated age or pathological load, suggesting that

these systems provide an approach to monitor highly compromised animals[72,156,157]. Furthermore, given the inter-institutional consistency of touchscreen performance[123,125] this apparatus could also enable characterisation of best practice via direct quantitative comparison of procedures across facilities.

As an example of this potential, cognitive bias (CB) behavioural tests have recently emerged as potential tools for assessing animal welfare. Cognitive biases have been widely studied in humans and reflect the manifestation of a negative or positive emotional state as a pessimistic or optimistic bias in information processing in conditions of uncertainty[158,159]. Critically, such emotionally-modulated cognitive processes can also be assessed in rodents and NHPs[95,96,158]. To date, CB tests have been used to evaluate the effects of changes in housing conditions[94], environmental enrichment[160], intraperitoneal dosing handling[161] and general handling methodologies[162] in rats, mice and birds. In many of these studies, animals are trained in a Go/No go task (where 'Go' requires a response to a rewarding stimulus and 'No go' requires withholding of a response to an unrewarding stimulus). After performance stabilization, ambiguous stimuli (which possess characteristics intermediate between the rewarding and unrewarding stimuli) are occasionally presented to the animals. The response pattern upon presentation of an ambiguous stimulus can then be used to infer the extent of any 'optimistic' or 'pessimistic' bias expressed by an animal and in turn the affective state of that animal.

Recent reports of CB assessments in animals indicated that rats housed in 'unpredictable' housing conditions exhibited a pessimistic bias (and therefore a negative emotional state) when compared to rats housed in 'predictable' conditions[94]. A CB task also demonstrated that provision of environmental enrichment enhanced positive emotional state (on the basis of the expression of 'optimistic' response bias to ambiguous stimuli) in birds[163]. Alterations in handling associated with injection of compounds was also manifest in CB performance[161]. Specifically, animals injected using conventional handling/restraint exhibited negative biases compared with those injected using a modified handling approach[161].

CB tasks can also be implemented in touchscreens and have shown potential for assessing welfare in NHPs[130]. While many CB tasks for animals have used tones as stimuli[164], visual stimuli can be used in touchscreens increasing the similarity of the task to that used in humans[165]. Indeed, a Go/No go touchscreen CB task was developed for NHPs using lines of different sizes to represent the Go ('CS+') and No go ('CS-') stimuli and lines of intermediate size as ambiguous stimuli. In this task, animals provided with environmental enrichment showed an optimistic bias. However, when the animals were tested after their statutory health

check (requiring restraint and ketamine hydrochloride injection), a pessimistic bias was observed[128]. A touchscreen task to evaluate CB through ambiguous cue interpretation has recently been devised for use in rodents[96] and although the effect of welfare manipulations on task performance remains to be determined, some evidence suggests that social experience impacts performance[97].

A further example of an affective state-targeted touchscreen task that could contribute to welfare assessment is the touchscreen implementation of the PR schedule. Based on the classical PR schedule[166], the touchscreen variants for mice[129] and rats[130] have been validated using pharmacological manipulations in non-touchscreen paradigms to bidirectionally impact performance as measured by the metric known as breakpoint (the maximum amount of physical effort in the form of screen touches an animal is willing to expend to obtain a palatable reward)[64,166–168]. Poor PR performance (defined by low breakpoint) is consistent with reduced motivation for reward. This behavioural outcome is interpreted as apathy or anergia-like behaviour and it is a pervasive and debilitating symptom common to many neurodegenerative and neuropsychiatric diseases[53].

Aside from rodent models of neuropsychiatric or neurodegenerative disease, lack of motivation or anergia/apathy in otherwise healthy animals is largely triggered by exposure to stressors. Similar to the CB construct, it is highly unlikely that an apathy-like phenotype could be identified from a routine visual welfare check. Routine blood sampling to determine plasma corticosterone levels for welfare monitoring purposes is also unnecessarily invasive, puts animals at risk of injury and exposes them to restraint and manipulation-induced stress that could confound any measurements taken. The PR task offers an ideal way to address these issues in that it leverages the merits of the touchscreen apparatus, offers a high sensitivity screening platform and, crucially, studies have shown that it is sensitive to exposure to elevated corticosterone levels using both non-touchscreen equipped[169,170] and touchscreen equipped behavioural chambers (Lopez-Cruz, personal communication[171]). While more limited in use to date than the CB task, non-touchscreen versions of PR have provided some potential insights into husbandry practices. For example, housing conditions can impact PR performance in rats (mixed-sex vs. single-sex holding rooms[172]) and in NHPs (single housing vs. pair-housing[173]). These studies support the viability of using the PR schedule as a tool to assess the effect of husbandry-related factors on laboratory animal welfare.

Taken together, these examples highlight the potential of touchscreen assays to be utilised as cross-species welfare assessment tools that are sensitive to many aspects of laboratory animal husbandry practice including housing conditions, environmental enrichment

and handling method. Given the high levels of standardisation and consistency offered by the touchscreen apparatus, this platform also offers opportunities for assessment of affective state longitudinally across the life of a laboratory animal and for quantitative comparison of procedures across facilities and institutions to identify best practice. Given that touchscreen versions of tasks targeting constructs related to mood and emotional state (e.g.[96]) would have high similarity to tasks used in humans[158], these assays could also be utilised as a more refined approach for evaluating mood-related symptoms in animal models and for screening compounds for antidepressant efficacy, replacing current methods like the FST and TST.

## Conclusions

The touchscreen behavioural assessment apparatus offers an increasingly large range of tasks to evaluate a wide variety of psychological constructs. The touchscreen method can yield significant 3Rs benefits, with potential implications for *Relative Replacement* as well as enhancements in both *Reduction* and *Refinement*, including the explicit avoidance of aversive stimuli, the possibility to utilise within-subject designs and the capacity to use a panel of assessments in the same cohort of animals. The standardisation of approach across laboratories will facilitate direct comparison of data and has already led to the development of touchscreen data sharing initiatives[115–117]. Furthermore, the high translational potential of this approach may increase the probability of promising findings from *in vivo* animal experiments being successfully implemented in the clinical context.

Touchscreen-based assays specifically targeting aspects of emotional state are now available[28,48,167,174]. They represent an opportunity to establish a quantitative approach to cage-side animal welfare monitoring and to characterise best practice approaches to laboratory animal husbandry and care.

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## Competing interests

The authors declare no competing interests.

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**Table 1|The 3Rs principles of animal research.**

| Principle | Definition |
|---|---|
| ***Full/Absolute Replacement*** | Any approach that results in the direct replacement of animals or the avoidance of their involvement in an experiment in which they would previously have been required. |
| ***Partial/Relative Replacement*** | Any approach involving organisms that are not, on the basis of current evidence, considered able to experience suffering or |

| | |
|---|---|
| | replacing the use of more sentient animals with animals that are lower on the phylogenetic scale. |
| ***Reduction*** | Any approach that reduces the number of animals involved in a particular study consistent with the scientific aims. Reduction can also include the enhancement of per animal data yields in a study, thereby eliminating any need for additional animals to be involved, and the sharing of data and resources between investigators. |
| ***Refinement*** | Any approach that minimises pain, suffering, distress or lasting harm and enhances animal welfare. |

Definitions adapted from [3,7,10,12,13,18,175,176]

## Box.1 | Touchscreen-equipped behavioural apparatus

In general, touchscreen-equipped behavioural apparatus consists of an operant arena housed within a sound-attenuating chamber that is equipped with a fan to provide ventilation and mask background noise. A touchscreen is mounted at one end of the arena and a reward collection magazine connected to a standard liquid or pellet dispenser is attached to the opposite wall as exemplified by the Bussey-Saksida touchscreen system. The touchscreen and associated devices are typically controlled by commercial software such as ABET II, K-Limbic or Whisker[30,177], with an increasing number of open-source solutions now becoming available[178–182]. Variations in design are dependent on manufacturers and species and can include variations in arena or touchscreen size, reward magazine capacity, arena shape (e.g., trapezoidal vs. cube/cuboidal) and floor type.

**[MPS: Please include Figure 1 as Figure box here. And find below the figure legend.]**

**Standard Bussey-Saksida touchscreen chambers. a,** the mouse and **b,** the rat systems show the differences in screen, arena size and floor type required to accommodate the different species (Campden Instruments Ltd, Loughborough UK).