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Measuring paw preferences in dogs, cats and rats: Design requirements and innovations in methodology

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ABSTRACT

Studying behavioural lateralization in animals holds great potential for answering important questions in laterality research and clinical neuroscience. However, comparative research encounters challenges in reliability and validity, requiring new approaches and innovative designs to overcome. Although validated tests exist for some species, there is yet no standard test to compare lateralized manual behaviours between individuals, populations, and animal species. One of the main reasons is that different fine-motor abilities and postures must be considered for each species. Given that pawedness/handedness is a universal marker for behavioural lateralization across species,

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this article focuses on three commonly investigated species in laterality research: dogs, cats, and rats. We will present six apparatuses (two for dogs, three for cats, and one for rats) that enable an accurate assessment of paw preference. Design requirements and specifications such as zoometric fit for different body sizes and ages, reliability, robustness of the material, maintenance during and after testing, and animal welfare are extremely important when designing a new apparatus. Given that the study of behavioural lateralization yields crucial insights into animal welfare, laterality research, and clinical neuroscience, we aim to provide a solution to these challenges by presenting design requirements and innovations in methodology across species.

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KEYWORDS Paw preference; behavioural laterality; species-specific; design requirements; methodological innovations

Introduction

Over the past decade, laterality research has made tremendous progress (Hausmann et al., 2021; Ocklenburg & Güntürkün, 2024). Laterality refers to the dominance of one hemisphere over the other while performing certain actions (Ocklenburg et al., 2021; Vallortigara & Rogers, 2020). Comparative laterality research has made much progress in recent years, yet it still encounters unresolved problems, particularly concerning reliability and validity (Ocklenburg et al., 2021). Here, innovations and more specialized approaches can help and facilitate the translation of findings between species. In general, there are different methods to assess both motor and sensory behavioural lateralization in mammals with different focuses dependent on the species, such as body- and head-turning asymmetry in rodents (Mundorf et al., 2020, 2021; Soyman et al., 2018), tail-wagging in dogs (Quaranta et al., 2007), and head-orienting responses to auditory stimuli in cats (Siniscalchi et al., 2016). Additionally, various techniques, including the orientation of the head towards not only auditory but also visual stimuli, have been studied (Siniscalchi et al., 2010, 2018). Furthermore, studies have investigated the asymmetric use of the nostrils in both dogs and cats (respectively, d'Ingeo et al., 2023; Siniscalchi et al., 2011), contributing to a comprehensive understanding of lateralized behaviours across different sensory domains in various mammalian species. One form to assess behavioural lateralization across species, including humans, is by measuring the preference for one limb, paw, or hand (Manns et al., 2021; Ocklenburg et al., 2019; Papadatou-Pastou et al., 2020). In humans, handedness is reflected as right-hand dominance in the general population (Papadatou-Pastou et al., 2020). Similar to humans, other animals also show functional asymmetries, which are mostly manifested as limb/paw preferences or other biased actions of appendages. This phenomenon is a shared characteristic among vertebrates and invertebrates (Frasnelli, 2013; Güntürkün et al., 2020; Ströckens et al., 2013). This article focuses on three distinct species—dogs, cats, and rats, which have been extensively studied in the field of laterality. Some studies on dogs, cats, and rats, including Tan (1987) for dogs, Wells and Millsopp (2009) for cats (noting opposite directions between males and females), and Güven et al. (2003) for rats, suggested the presence of asymmetry at the population level. In contrast, other studies, such as Marshall-Pescini et al. (2013) for dogs, Pike and Maitland (1997) for cats, and Cunha et al. (2017) for rats, presented conflicting findings. To illuminate contradictory findings, systematic meta-analyses on paw preferences in dogs, cats, and rats have been conducted. These studies reveal that asymmetry for paw preference at the individual level is a common trait among these three species. In contrast, unlike hand preference in humans, asymmetry for paw preference is not observed at the population level (Manns et al., 2021; Ocklenburg et al., 2019).

Domestic dogs (*Canis familiaris*) and humans have a long common evolutionary history of mutual benefit and cooperation (Thalmann et al., 2013). This common evolutionary past has caused dogs to become dependent on humans for living and to develop some anatomical and functional expression features only for communication with humans (Kaminski et al., 2019). A very recent investigation has revealed that the hand preferences of owners could influence their dogs' paw preferences (Charlton & Frasnelli, 2023). Moreover, humans and dogs have many common behaviours linked to psychiatric disorders with the same pathological mechanisms (Moon-Fanelli & Dodman, 1998; Overall, 2000; Starkey et al., 2005). A recent study has shed light on the correlation between facial asymmetry in dogs and aggression- and fear-related disorders, drawing parallels with similar patterns observed in the human species (Siniscalchi et al., 2022). For this reason, dogs are considered an promising model for humans to understand the underlying mechanisms of psychiatric disorders (Vermeire et al., 2012). Another reason for the increase in research interest in dogs is that dogs are naturally cooperative and trainable models that are easily motivated when working with humans (Dobos & Pongrácz, 2023). In other words, the preparation of a dog for an experiment is easier than that of other species. Considering their evolutionary history and integration with the human social environment, dogs and humans have a number of common basic behavioural and functional characteristics and skills (Miklósi & Topál, 2013). Considering the corresponding functions of these common skills, dogs are a reliable species for investigating certain features of human social cognition evolution in comparative neuroscience (Berns & Cook, 2016). Lateralization studies in dogs are important not only for understanding humans or the evolutionary processes of lateralization but also for dog welfare (Simon et al., 2022b; Siniscalchi et al., 2017; Wells, 2021). The strong literature data suggesting that acute and chronic stress alter functional cerebral asymmetry (FCA) are promising that well-being can be evaluated by measuring FCA in dogs (Salgirli Demirbas et al., 2019, 2023). Moreover, the detection of animal welfare by non-invasive methods is of great importance in the field of veterinary medicine.

Domestic cats (*Felix catus*) have been companions to humans for about 10,000 years. The longstanding relationship between domestic cats and humans has resulted in the development of shared behavioural traits (Fugazza et al., 2021) and distinctive human-specific vocalizations in cats (Bradshaw, 2016). Domestic cats also demonstrate the ability to form and sustain social connections, extending not only to humans but also to other types of pets. Despite the social characteristics of domestic cats, they still share the same behavioural features related to their hunting motivation as their ancestors. Domestic cats are, thus, still considered natural hunters (Turner, 2013). Theoretically, cats do not need to hunt for food since they can easily access food their owners provide. The innate feeding behaviour of cats requires hunting (prey seeking, stalking, and chasing) and scavenging (Landsberg et al., 2011), resembling their ancestral African wildcat (Rodan & Heath, 2015). This type of hunting behaviour requires complex motor skills to catch and consume prey successfully; therefore, effective paw usage is extremely important for cats. Furthermore, very recently, lateralized cats were reported to be more successful than ambilateral ones in problem-solving tests (Isparta et al., 2020). This finding supports the idea that having a clear preference for one paw provides an evolutionary advantage (Rogers, 2000) in cats as well. Taken together, the investigation of lateralization in cats is crucial in order to understand cat biology and is also interesting to shed light on the evolution of human laterality. In addition to all these, the results of a study conducted on different cat breeds perceived as emotionally non-reactive (Ragdoll and Maine Coone) and emotionally reactive (Persian and Bengal) by veterinary practitioners, suggested that measuring paw preference may be a valuable method for assessing emotional reactivity in domestic cats. This finding could contribute to our currently limited tools for determining breed-specific profiles, thereby leading to more successful cat-owner relationships (Wells & McDowell, 2019). Therefore, the development of reliable and reproducible methods for measuring paw preference in cats will also contribute greatly to animal welfare research.

Rats and mice are one of the most used vertebrate species in animal research worldwide (Baumans, 2005). For a long time, the reaching ability of rats and mice have been utilized as a behavioural paradigm in various scientific domains, including the examination of neural control of the forelimb (Kawai et al., 2015; Whishaw et al., 2008), assessment of brain damage (Alaverdashvili et al., 2008; Klein et al., 2012) functional recovery of forepaw function following neural injury (Ramanathan et al., 2006; Whishaw et al., 2008), evaluation of motor function in neurological disorders like Parkinson's disease (Klein & Dunnett, 2012), and investigation of laterality (Collins, 1968; Whishaw, 1992). Moreover, Whishaw (1992) suggested that rats and humans have similarities in their movements and the sequencing of their movements while reaching for food. A more recent study has supported this finding by revealing that rats' reach and grasp movements are strikingly similar to human's (Klein & Dunnett, 2012). Here, it is crucial to state that reaching tasks were originally utilized to measure paw preferences in mice (Collins, 1968). Since paw preference in rats is linked to cerebral asymmetry at the functional (Hamani et al., 2010), molecular (Zimmerberg et al., 1974), and behavioural levels (Castellano et al., 1989; Soyman et al., 2015), it has been considered the most apparent functional expression of cerebral asymmetry in rats, like handedness in humans. Assessing behavioural laterality in rats and mice can render important insights into biomedical investigations (Manns et al., 2021), mental health and consequences of stress exposure (Mundorf et al., 2020), neurodevelopmental alterations (Alonso et al., 1991; Mundorf et al., 2021), and psychiatric disorders (Ecevitoglu et al., 2020; Mundorf & Ocklenburg, 2023; Soyman et al., 2018). Additionally, the study of rodent models of psychiatric and neurodegenerative disorders has significantly advanced the knowledge of hemispheric asymmetries on the neurobiological level (see, for example, Mundorf & Ocklenburg, 2023; Ocklenburg et al., 2022).

In humans, handedness can be measured through two main ways: questionnaires (Oldfield, 1971) or behavioural performance measures (Annett, 1976), and can be characterized by strength and direction. Several different methods have been developed and utilized in dogs, cats, and rodents to measure behavioural laterality. To determine paw preferences in dogs, a variety of methods have been reported, including reaching for food (e.g., Aydinlioğlu et al., 2000; Laverack et al., 2021; Salgirli Demirbas et al., 2023), reaching for a toy (e.g., Charlton & Frasnelli, 2023; Duncan et al., 2022), paw-shaking (e.g., Wells, 2003), removal of tape from the eyes (e.g., Tan & Caliskan, 1987) or the nose (e.g., Batt et al., 2009; Quaranta et al., 2004); removal the blanket from the head (e.g., Wells, 2003), first stepping (the first foot placed forward) (e.g., Tomkins et al., 2010; Wells et al., 2018); stepping over a small hurdle (the first step) (Simon et al., 2022a); stabilization of a ball/object (e.g., Poyser et al., 2006); or paw used to hold a Kong toy (e.g., Barnard et al., 2017; Branson & Rogers, 2006; Marshall-Pescini et al., 2013; Salgirli Demirbas et al., 2019, 2023; Schneider et al., 2013). The methodologies for assessing paw preference through food retrieval tasks vary significantly across these studies. Laverack et al. (2021) encouraged dogs to use their paws to retrieve treats from a plastic or cardboard tube. Duncan et al. (2022) opted to place food under furniture for retrieval. Meanwhile, Salgirli Demirbas et al. (2023) implemented a stable, transparent, and adjustable apparatus specifically designed for assessing paw preference.

Several approaches have been employed to assess the paw preference of cats. The majority of these approaches consist of reaching tasks, which have become an increasingly common and effective method for assessing motor laterality. A few of these investigations required the cat to reach/

manipulate a moving object rather than a food-based reward, such as a suspended/rolled object (Wells & Millsopp, 2009) or a moving spotlight (Lorincz & Fabre-Thorpe, 1996). The majority of studies conducted in this particular field have been focused on food-reaching tasks, wherein the cat needs to retrieve a food reward from an object positioned right in front of them (Cole, 1955; Duncan et al., 2022; Graystyan & Molnar, 1954; Isparta et al., 2020; McDowell et al., 2016; Pike & Maitland, 1997; Reiss & Reiss, 1998; Wells & Millsopp, 2009). On the other hand, in some older studies, the testing procedure was performed on cats placed in a testing device such as the Wisconsin General Testing Apparatus (WGTA), a modified version of WGTA, or a special cage (Forward et al., 1962; Olmstead & Villablanca, 1979; Yetkin, 2002). In addition to the conventional food-reaching test paradigm, (McDowell et al., 2018) employed experimental paradigms involving initial stair descent and stepping over while entering the litter to evaluate the paw preferences of the cats.

The investigation into the phenomenon of behavioural lateralization in rodents dates back to the 1930s when Tsai and Maurer (1930) carried out the first study using the food-reaching test approach. Subsequently, the prevailing methodological approach in the literature has consistently involved the utilization of the food reach test methodology, such as the Collins' Test (Collins, 1968, 1969, 1975), Lateral Paw Preference Test (Waters & Denenberg, 1991), and Pawedness Trait Test (PaTRaT) (Cunha et al., 2017). Although many other testing apparatuses have been developed to determine paw preference (Güven et al., 2003; Tang & Verstynen, 2002), their basic working principle is the same: requiring animals to use their paws to reach food rewards. Moreover, Ballermann et al. (2001) proposed that the Pasta Matrix Reaching Test, designed to examine the reaching ability of animals more extensively, which involves the extent of the forelimb movement and ability to reach in a wide range of directions and distances, can also be used in laterality research.

Researchers often face methodological challenges while measuring laterality in animals. One of the main challenges in the field is that although validated tests exist for some species, such as rats and mice, to test pawedness (Collins, 1968, 1969, 1975), there is no standard test to compare lateralized manual behaviours between individuals, populations, and animal species. Standardized tests have the potential to promote scientific consistency in laterality research, allowing researchers to replicate studies more easily, and contribute to the cumulative nature of scientific knowledge. However, possible problems with standardized tests are anatomical differences (e.g., forelimb and paw anatomy) which hinder the comparability between species. Therefore, it is important to design apparatuses that take into account both species-specific requirements and standardized measures of laterality behaviour. Consequently, the designs show small differences to match the species but still allow for a standardized measure of laterality behaviour. For example, although a pegboard test is a good way to assess the hand performance of humans, it is difficult to adapt it to rhesus monkeys. Movement assessment panels (MAPs), on the other hand, can be applied to humans and rhesus monkeys more easily (Gash et al., 1999), but this test is not applicable to dogs or cats. Moreover, different test designs should be used for cats and dogs, although both are quadrupedal. Domestic cats use their paws more skilfully, given that, as skilled hunters, they use their paws during the hunt. Domestic dogs, on the other hand, are scavengers and thus only use their paws for holding objects or digging, behaviours that may be driven by various motivations. Thus, they do not need improved fine motor skills to manipulate objects with their paws. Therefore, employing standardized tasks across different animal species may potentially compromise the reliability of the obtained results (Lambert, 2012).

Another discussion on motor laterality tests is related to the one- and two-handed/pawed actions needed for solving the test. One of the common methods to test handedness in primates is simply reaching for food, which is an unimanual action requiring only one hand to perform a task. However, simple reaching during feeding is not considered an optimal measurement method since the results may change depending on the posture and positioning of the animal. The animal also does not need to use the dominant hand to reach for the food, which is in a position that it can easily take (Hopkins, 2006). On the other hand, coordinated bimanual manipulation-based tasks require using both hands, such as holding the object with one hand (non-dominant one) and manipulating the object with the opposite hand (dominant hand). Thus, fine motor skills should be used while performing those kinds of tests (Meguerditchian et al., 2010). According to the complexity hypothesis by Fagot and Vauclair (1991), primates show a weaker degree of handedness in simple feeding tasks in comparison to bimanual manipulation-based tasks (Lambert, 2012). Thus, the tasks required for bimanual actions are considered to be more sensitive and reliable for measuring hand dominance (Hopkins, 2006; Lambert, 2012). Since individuals must take a bipedal or seated posture during bimanual actions to maintain the freedom of both hands, postural factors that may affect the results are minimized (Roney & King, 1993). Indeed, bimanual manipulation tasks can be performed by some species, such as primates, rats, and mice, as they can use both hands/paws when sitting or standing on their rear ends. However, these tests are not applicable to quadrupedal animals such as dogs and cats. Although the ability of dogs and cats to use paws is different from each other, as mentioned above, their bimanual activity abilities are quite limited, unlike humans and primates. Therefore, it is not possible to apply complex bimanual performance tests to these animal species.

Lateralization in these three species, i.e., dogs, cats, and rats, has yet to be well understood, and existing studies in general were often statistically underpowered due to their sample sizes (Manns et al., 2021; Ocklenburg et al., 2019). One reason might be that the existing tasks to assess behavioural lateralization are labour-intensive and include long training and testing sessions. Moreover, studies vary greatly in study design and methodology. As a result, studies report conflicting results for the degree and direction of paw preference for these three species (Manns et al., 2021; Ocklenburg et al., 2019). Therefore, the goal of future species-specific tests is to develop tests that are (i) easy to implement by the researcher, (ii) easy to solve by the animal, and (iii) maximally standardized. This will enable experimenters to test more animals in a shorter time and, most importantly, to obtain valid and robust results across studies by increasing the statistical power.

In light of the research and literature knowledge carried out to date, it can be suggested that it is impossible to develop a single standard method to test laterality in all animal species. However, it is possible to develop tests with similar logic and purpose, considering the specific needs of the concerned species.

The primary objective of this article is to discuss the design criteria necessary for creating a standardized food-reaching test proposed as a recommended method for measuring hand preference. This discussion takes into consideration the species-specific characteristics of cats, dogs, and rats. Furthermore, the study aims to introduce novel species-specific food-reaching tests developed by the authors. The presented food-reaching tests (FRTs) can be used to assess the paw preferences of the animals as non-invasive and standardized evaluations that can be applied to animals without additional stress and any external intervention. The design criteria and the developed FRTs presented in this study were assumed to shed light on the methodologies to be developed for the field of animal laterality research in the future.

Design requirements for measuring behavioural laterality

Zoometric fit

Dogs

To ensure that the test encourages the animal to use its paw instead of its head or tongue, it is essential to restrict the slot where the paw is inserted. Given variations in body sizes among breeds and age groups, the height of the slot should be vertically adjustable, and the place where the food is dropped should be adapted to accommodate different animal sizes.

Cats

The purpose of designing standard apparatuses is to allow the cats to manipulate the target conveniently, regardless of the difference in size between breeds. All three apparatuses for testing cats are applicable to any cats older than six months.

Rats

The main purpose of designing an adjustable apparatus is to allow animals to be tested at different developmental stages, regardless of their body size. Moreover, combining three different motor laterality tests within a single testing apparatus is also useful to reduce the habituation time of the rat individuals for each test.

Research reliability considerations

The equipment should enable visibility of the animals' actions for the researcher's observations and video recording purposes. There should be consistency in where the food is dropped within a test to maintain the reliability of the measurement. The animal needs to be motivated to retrieve the food. Therefore, all animals should be able to smell the food through holes and also got a free treat before the test. The test setup should attract the interest of the animal to be able to complete the task. Therefore, the food should be easily noticeable to the animals. To maintain the accuracy of the measurement, the experimenter's presence and actions should not distract the animals, especially during the food-dropping process, nor be a fearful stimulus. In addition, the equipment and test setup can also be specifically modified according to species. For instance, considering the differences between dog breeds, the inclusion of varying levels of difficulty in the food-reaching task can be effective in encouraging the use of paws and observing targeted paw interaction. To provide a more comprehensive depiction, one should consider the distinct lengths of forelimbs observed in large breeds like the Anatolian Kangal dog and Saint Bernard, which markedly differ from those in small breeds such as the Yorkshire Terrier and Pomeranian. This variation in lengths of forelimbs contributes to differences in the level of task complexity, depending on the placement of food rewards for these dog breeds. The different difficulty levels of the dog food reaching tests also provide an advantage in motivating the dog to work for the food reward.

Robustness considerations

Since dogs and cats exert force in test conditions and thus push and pull the equipment during the test, it needs to be structurally stable to guarantee measurement consistency and observability. The rodent task also requires stability to prevent the rats from getting anxious about a potentially moving apparatus due to the movement of the rodent inside the box. Consequently, the testing equipment needs to endure these rough conditions throughout the research.

Maintenance considerations

Generally, food access tests require measurement over a period of time. In this period, the paw movements should be counted repetitively multiple times; ideally, the food should be automatically dropped to allow a standardized and non-biased procedure. As the paw movement has to be counted many times, several pieces of food should be quickly available during the test. Therefore, multiple food pieces can be stored within or next to the equipment. The equipment is to be used throughout a time period, and different types of food may be used in the feeder unit; thus, the parts storing and presenting the food should be easily accessible for cleaning (i.e., to prevent possible mould and bacteria formation, and also remove odours). It should be easy to carry the equipment to different research settings.

Animal welfare

As per Article 1 of the UK Farm Animal Welfare Council and the present European (EU) legislation on animal welfare (Farm Animal Welfare Council, n.d.) "*Freedom from hunger and thirst by ready access to fresh water and a diet to maintain full health*", we support that no drink or food restrictions should be administered in the experiments. The reliability of tests performed by depriving animals of their physiological needs is an issue that the scientific world has been discussing for years. When we test animals under negative conditions, such as food or water deprivation, we are not testing their neutral state. For this reason, our main goal was to prepare a test setup in which the animals could be motivated enough without any food or drink restrictions. The experimental setups outlined in this article go beyond addressing just the freedom from hunger and thirst; they comprehensively consider the entire spectrum of the five freedoms. This encompasses freedom from pain, injury, and disease, as well as freedom from discomfort, fear, and distress. Additionally, our approach ensures that animals have the freedom to perform normal and natural behaviours. Dogs and cats should not be caged or constrained in order not to further increase their stress level. Rats performed habituation with the apparatus during two days before the first experiment to avoid stress-related behaviour. The apparatus should not be too large since it can also lead to stress-related behaviours or anxiety. It is important to note that the testing session should be interrupted when clear signs of stress are displayed by the animal. No bedding should be placed in the apparatus to create a neutral environment.

The equipment should not harm the animals' physical, mental, and cognitive health while guaranteeing no fear/anxiety/frustration that leads to protective motivational-emotional systems. Any kind of chewing-encouraging parts and/or material of the apparatus should be avoided. Plexiglass and plastic materials, as well as varnished wood, are proper materials for easy cleaning and are non-harmful. In adherence to the Five Freedoms outlined in Article 1 of the UK Farm Animal Welfare Council and current EU legislation, we prioritized these principles in our study, ensuring a comprehensive focus on animal well-being.

Proposed food-reaching tests for laterality measurement

Considering the design requirements for measuring the paw preferences of these species, the present paper describes designing species-specific, standardized food-reaching-based tests for these three species (Table 1).

Dog

To determine the paw preferences of dogs, a testing setup with an automatic system consisting of two components and a manual testing apparatus has been developed (Figure 1) (Supplementary Video 1, 2; which can be accessed at https://osf.io/qne2r), as utilized in our recent study (Salgirli Demirbas et al., 2023). The apparatus featured five distinct levels, each set at equal distances, to accommodate the varying forelimb lengths across dog breeds—a necessity for assessing precise paw use. Having different levels of positions was also practical to motivate the dog to work for the food reward. The FRTs in this study determined 31.9% of dogs to be left-pawed, 27.7% to be right-pawed, and 40.4% to be ambilateral.

Procedure

To motivate and habituate the dogs with the testing apparatus, the experimenter offered small food rewards as an introductory step at the beginning of each session. The test session mostly started at the easiest level (level 1) to motivate the dog with food, and the level was adjusted depending on the dog's motivation and forelimb lengths. For large breeds, the furthest distances, levels 4 and 5, were used, while medium-sized dog breeds were tested on levels 3 and 4, and small breeds on levels 1 and 2. As training may cause a bias toward certain paw use, none of the dogs received any training before and/or during the study. There were also no food restrictions for the dogs during the study. Dogs were

Table 1. Design requirements and specifications for measuring paw preference in dogs, cats, and rats.

	Design Requirement	Species	Task	Design Specification
Zoometric fit	Adjustability for different sizes	Dogs	Automated FRT	The slot is vertically adjustable and the food can be dropped at 5 different levels.
			Manual FRT	
		Cats	Easy Lid Opening Test	Compatible for all sizes, no adjustability needed.
			Complex Lid Opening Test	
			Simple FRT	
		Rats	Collins Paw Preference Test	The height of the middle layer in the apparatus is vertically adjustable.
			Head-turning Asymmetry Test	
			Pasta Matrix Reaching Test	Adjustable pasta unit is compatible for all sizes/age groups.
Research reliability considerations	Ease of observation	Dogs	Automated FRT	Transparent plexiglass material is used in all parts of the test equipment.
			Manual FRT	
		Cats	Easy Lid Opening Test	The cats could be observed easily since they were not in a container. The test equipment is partly contained of transparent plastic except the wooden bottom and dark lids (for complex lid opening test).
			Complex Lid Opening Test	
			Simple FRT	The cats could be observed easily since they were not in a container.
		Rats	Collins Paw Preference Test	Transparent plexiglass material is used in all parts of the test equipment except the middle layer.
			Head-turning Asymmetry Test	
			Pasta Matrix Reaching Test	Transparent plexiglass material is used in all parts of the test equipment.
	Consistency in position of the target reach	Dogs	Automated FRT	Each time the food is dropped on an exact position and a sliding mechanism pushes it to a designated place from the available 5 levels by remote control.
			Manual FRT	Each time the food is dropped on an exact position and a sliding mechanism was manually pushed to a designated place from the available 5 levels.
		Cats	Easy Lid Opening Test	The food is placed under the cups on the wooden block.
			Complex Lid Opening Test	The food is placed into the cups.
			Simple FRT	The food is placed equidistantly to the left and right walls inside of the apparatus.

(*Continued*)

Table 1. Continued.

Design Requirement	Species	Task	Design Specification
	Rats	Collins Paw Preference Test	The food is placed in the narrow tube, which was placed exactly equidistantly to the left and right wall of the front wall of the chamber.
		Head-turning Asymmetry Test	The dispenser with sugar-water was placed equidistantly to the left and right wall of the chamber on the top of the apparatus.
		Pasta Matrix Reaching Test	The pasta pieces were placed on a rack with an array of holes, 13 rows deep by 11 rows wide. The rack was placed before a 1 cm wide hole on the front of the apparatus.
Motivation	Dogs	Automated FRT Manual FRT	There are 5 levels of positions for dropping the food: starting from the one which is closer to the slot of the farthest one.
	Cats	Easy Lid Opening Test Complex Lid Opening Test	The first treat is dropped on the wooden surface by the experimenter.
		Simple FRT	The first treat is placed in front of the hole of the apparatus by the experimenter.
	Rats	Collins Paw Preference Test	The first treat is dropped through the inserted tube by the experimenter. Both rats and experimenter can access the tube easily from each side.
		Head-turning Asymmetry Test	By placing the dispenser in its position, a few drops of sugar-water fall down towards the rat.
		Pasta Matrix Reaching Test	The pasta rack topped with hazelnut cream is placed in front of the hole by the experimenter. Both rats and experimenter can access the rack easily from each side.
Attractiveness	Dogs	Automated FRT Manual FRT	Transparent plexiglass is used to make food easily seen.
	Cats	Easy Lid Opening Test Complex Lid Opening Test	Transparent plastic cups are used to make food easily seen. The holes in the cups/lids also allow rats to smell the treats.
		Simple FRT	Transparent plexiglass is used to make food easily seen.
	Rats	Collins Paw Preference Test Head-turning Asymmetry Test Pasta Matrix Reaching Test	Transparent plexiglass is used to make food easily seen. The holes in the apparatus also allow rats to smell the treats.
Unobtrusiveness	Dogs	Automated FRT	During the test, the food is dropped and level adjustments can be made by using a digital remote controller.
		Manual FRT	During the test, the food is dropped and level adjustments can be manually made.
	Cats	Easy Lid Opening Test Complex Lid Opening Test	Before the test, the treat is manually placed in the right position.
		Simple FRT	During the test, the food is placed manually into the apparatus.

		Rats	Collins Paw Preference Test	During the test, the food is placed manually into the tube.
			Head-turning Asymmetry Test	Before the test, the treat is manually placed in the right position.
			Pasta Matrix Reaching Test	
Robustness considerations	Structural stability	Dogs	Automated FRT	Since the plexiglass material is heavy itself it is stable enough, so that the dogs could not move it.
			Manual FRT	
		Cats	Easy Lid Opening Test	Plastic cups were attached to the wooden block with cable ties and stabilized with tiny blocks on each side. The bottom of the block was covered by anti-slippery material.
			Complex Lid Opening Test	Plastic cups were screwed to the wooden block and the lids were attached to the cups by cable ties. The bottom of the block was covered by anti-slippery material.
			Simple FRT	The plexiglass material is heavy itself and anti-slippery covering was attached in the corners at the bottom of the apparatus.
		Rats	Collins Paw Preference Test	Since the plexiglass material is heavy itself it is stable enough, so that the rats could not move it while sitting in the box.
			Head-turning Asymmetry Test	
			Pasta Matrix Reaching Test	
	Durability	Dogs	Automated FRT	10 mm thickness is preferred for the plexiglass material.
			Manual FRT	
		Cats	Easy Lid Opening Test	The task contained a wooden surface with adjusted plastic cups.
			Complext Lid Opening Test	
			Simple FRT	10 mm thickness is preferred for the plexiglass material.
		Rats	Collins Paw Preference Test	10 mm thickness is preferred for the plexiglass material.
			Head-turning Asymmetry Test	
			Pasta Matrix Reaching Test	

(*Continued*)

Table 1. Continued.

	Design Requirement	Species	Task	Design Specification
Maintenance considerations	Ease of feeding	Dogs	Automated task	Remotely operated feeder unit, which automatically drops the food, is installed. In case the automatic feeder malfunctions, a manually operated feeder unit is provided.
			Manual task	A manually operated feeder unit is provided.
		Cats	Easy Lid Opening Test	
			Complex Lid Opening Test	
			Simple FRT	
		Rats	Collins Paw Preference Test	
			Head-turning Asymmetry Test	Before the test, the treat is manually placed in the right position.
			Pasta Matrix Reaching Test	
	Quickness in feeding	Dogs	Automated FRT	A food storage is provided within the equipment, from where the food is dropped.
			Manual FRT	An extern food storage is provided, from where the food is taken to insert manually.
		Cats	Easy Lid Opening Test	
			Complex Lid Opening Test	
			Simple FRT	
		Rats	Collins Paw Preference Test	
			Head-turning Asymmetry Test	Before the test, the treat is manually placed in the right position.
			Pasta Matrix Reaching Test	
	Ease of cleaning	Dogs	Automated FRT	Plexiglass surfaces are used to be cleaned.
			Manual FRT	
		Cats	Easy Lid Opening Test	Plastic cups are used to be cleaned.
			Complex Lid Opening Test	
			Simple FRT	Plexiglass surfaces are used to be cleaned.
		Rats	Collins Paw Preference Test	
			Head-turning Asymmetry Test	
			Pasta Matrix Reaching Test	

	Transportability	Dogs	Automated FRT	The equipment is composed of two units to ease carrying.
			Manual FRT	The equipment is small and composed of only one unit.
		Cats	Easy Lid Opening Test	
			Complex Lid Opening Test	
			Simple FRT	
		Rats	Collins Paw Preference Test	The equipment is composed of one main unit and adjustable units for Collins and pasta matrix or head turning test to ease carrying and flexibility.
			Head-turning Asymmetry Test	
			Pasta Matrix Reaching Test	
Animal welfare	Noninvasiveness	Dogs	Automated FRT	The equipment is standalone and free from any harmful materials to animal health. There is no need for any food/beverage restrictions, even for short durations.
			Manual FRT	
		Cats	Easy Lid Opening Test	
			Complex Lid Opening Test	
			Simple FRT	
		Rats	Collins Paw Preference Test	
			Head-turning Asymmetry Test	
			Pasta Matrix Reaching Test	

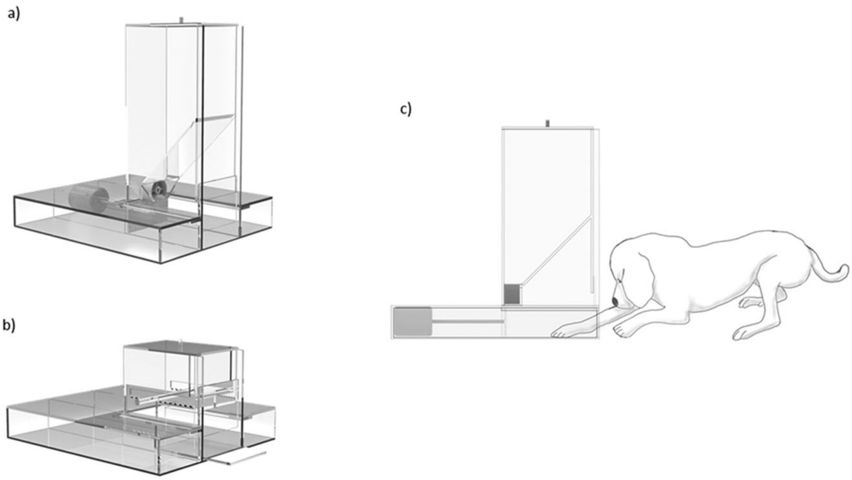

Figure 1. Automated food-reaching test (a); manual food-reaching test (b) for dogs; and a dog performing the automated food-reaching test (c).

always allowed to roam/move freely in the testing arena; they were not on a leash. The dogs' owners were also present in the testing room/environment throughout the testing sessions to avoid any potential stress caused by separation from the owner. However, positive reinforcements such as social (e.g., verbal praise) or tactile (e.g., petting) interactions by the experimenter/owner were explicitly avoided during testing to provide a non-biased paw preference for the dogs. The experimenter always sat behind the testing apparatus to avoid distracting the dog and only initiated a new session if the animal successfully reached and received the food in the apparatus. Each dog was tested individually in the testing area. The testing session was terminated when the dogs lost their attention to the experiment or reached 50 paw responses, which the experimenter counted as real-time.

Cat

Two novel testing apparatuses, namely the Easy Lid Opening Test and Complex Lid Opening Test, were designed for cats and can be used to measure both problem-solving skills and paw preference (Figures 2 and 3) (Supplementary Video 3,4: which can be accessed at https://osf.io/qne2r). Additional detailed information about these paw preference tests, which are also considered problem-solving tests, can be found in our previous study referred to as Test 1 and Test 2 (Isparta et al., 2020).

In this study, Easy Lid Opening Test determined 31.5% of cats to be left-pawed, 26.3% to be right-pawed, and 42.10% to be ambilateral; while

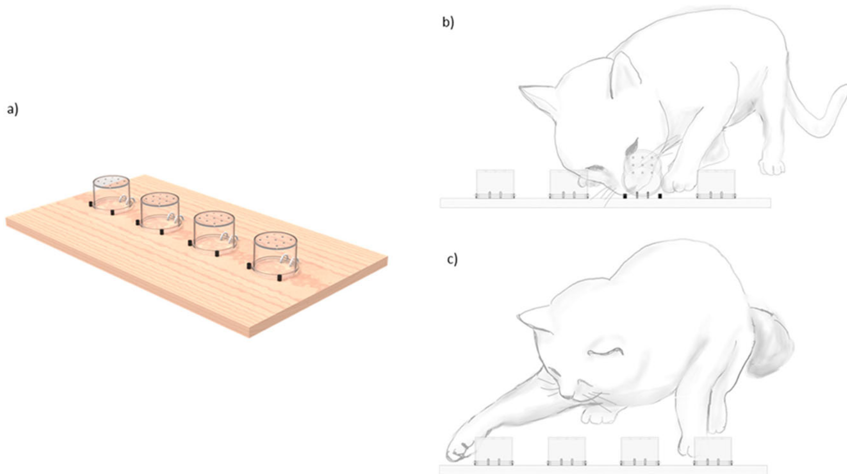

Figure 2. Easy Lid Opening Test for determining the paw preferences of the cats (a); a cat performing Easy Lid Opening Test using its mouth, nose, and/or head (b); and a cat performing Complex Lid Opening Test using its paw (c).

Complex Lid Opening Test identified 37.5% of cats as left-pawed, 27.5% as right-pawed, and 35% as ambilateral. In addition to these two testing apparatuses, a novel simple food-reaching testing apparatus to measure the paw preferences of the cats was also designed in this article (Figure 4) (Supplementary Video 5, which can be accessed at https://osf.io/qne2r).

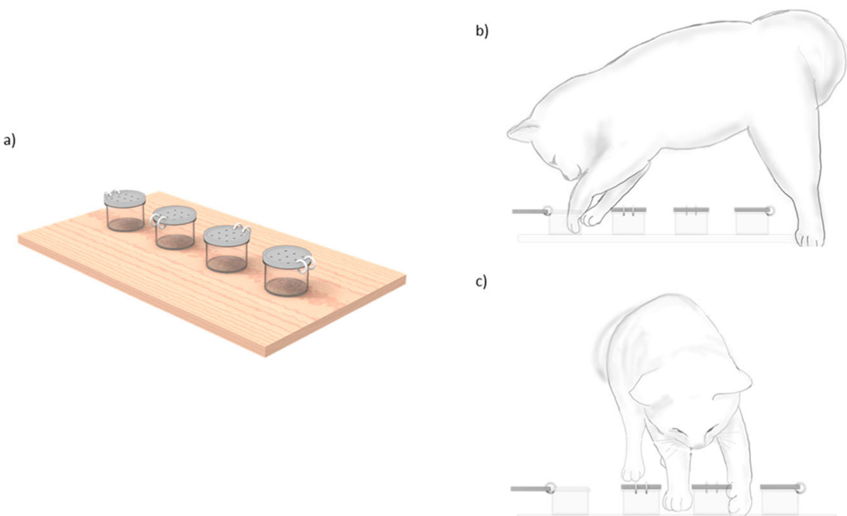

Figure 3. Complex Lid Opening Test for determining the paw preferences of the cats (a); a cat using its paw to reach for food after the lid is opened (b); and a cat using its paw to open the lid (c).

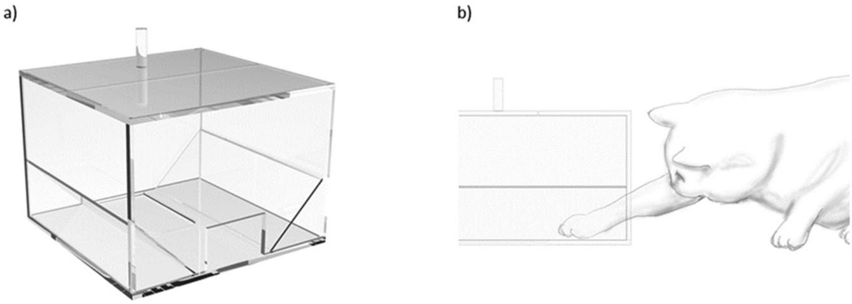

Figure 4. Simple Food-reaching Test for determining the paw preferences of the cats (a); a cat performing the test (b).

Procedure

The detailed procedure of cat problem-solving tests can be found in Isparta et al. (2020).

For the new cat food reaching task, cats did not receive any training beforehand. Cats were first motivated with a free treat, and no food restriction was performed. Only cats with good social interaction and positive emotional states were tested. During the experiment, the owner/experimenter stayed in the same room with the tested cat behind the apparatus, whereas no other cats were present simultaneously. The experiment started with the experimenter/owner placing a treat manually in the middle of the apparatus. Every time the cat reached the treat, another treat was immediately placed in the middle of the apparatus again. Each cat was tested individually in the testing area. The testing session was terminated when the cats lost their attention to the experiment or reached 50 paw responses, which the experimenter counted in real-time.

Rat

A novel testing apparatus was designed by combining three different motor laterality tests, including the Pasta Matrix Reaching (Ballermann et al., 2001), Collins Paw Preference Test (Collins, 1968), and Head-Turning Asymmetry Test (Soyman et al., 2018) (Figure 5) (Supplementary Video 6,7,8: which can be accessed at https://osf.io/qne2r). This testing apparatus was successfully used to assess paw preference of the rats in the doctoral dissertation authored by Isparta (2023). Within the scope of this doctoral thesis, the Collins test classified 41.1% of the adult rats to be left-pawed, 35.2% to be right-pawed, and 23.52% to be ambilateral, whereas the Pasta Matrix Reaching Test determined 41.9% as left-pawed, 48.3% as right-pawed, and 9.6% as ambilateral.

Given the unique situation that experimental rodents drink through a bottle, they can be forced to turn their heads to access the water dispenser simply by the placement of the water dispenser (Soyman et al., 2018). As left-sided head-

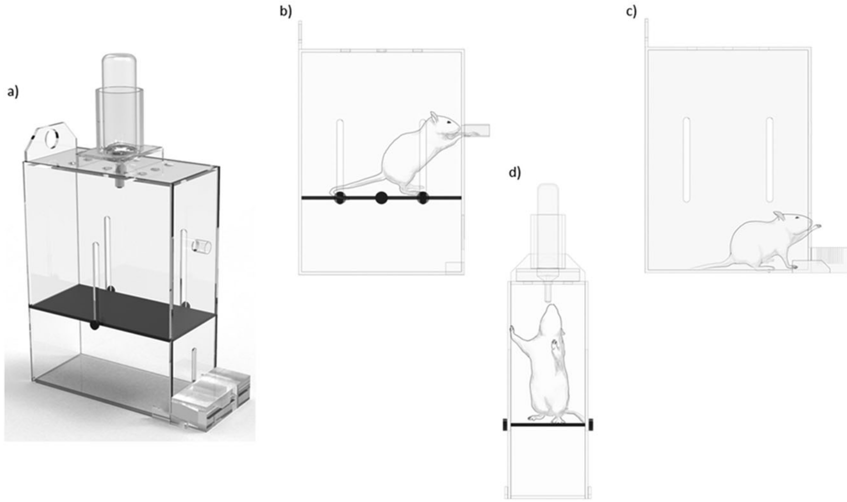

Figure 5. Motor laterality testing apparatus by combining the Pasta Matrix Reaching Test (Ballermann et al., 2001), the Collins Paw Preference Test (Collins, 1968; 1969; 1975), and the Head-Turning Asymmetry Test (Soyman et al., 2018) for rats (a), a rat performing the Collins Paw Preference Test (b), a rat performing the Pasta Matrix Reaching Test (c), and a rat performing the Head-Turning Asymmetry Test (d).

turning asymmetry has been associated with increased despair behaviour in rats (Ecevitoglu et al., 2020; Soyman et al., 2018) and can thus render important insights into mental health, the developed apparatus includes this measure.

Further information is provided for each species and task, considering factors such as zoometric fit, research reliability, material robustness, maintenance, and animal welfare during and after testing. Ideally, all requirements and specifications should be met when designing new apparatuses (Table 1).

Procedure

General advice

Advice 1

We highly recommend performing behavioural tests during the dark phase of a 12/12 h light/dark cycle since their vision systems are much more adapted to the low-light conditions they are mostly exposed to in the wild compared to the brighter light conditions (Balcombe, 2010).

Advice 2

We recommend taking animals to the experimental room/area for at least 30 min prior to the test session allowing them to adapt to the new environment. Therewith, potential stress-related influences on behaviour can be excluded.

Advice 3

The experimental room should consist of non-disturbing conditions, such as noise and foreign smell, for the animal during the test sessions. Each animal needs to be tested individually in the test apparatus in the experimental room. The testing setup needs to be cleaned after each testing session.

Advice 4 (habituation step)

We used a smaller version (11 × 12.5 × 21.5 cm) of the Collins test apparatus as a demo for habituating the animals to the plexiglass material. The apparatus was placed in their home cages in the experimental room for 15–30 min two days before the behavioural tests were performed allowing animals to get familiar with the apparatus in general and to lose their potential fear of the novel stimulus. Food reward was placed in the testing box but not in the tube for the Collins test to enable them a first taste of the reward without developing any side preferences.

Collins paw preference test

After the test apparatus had been adjusted according to the size of the subject (e.g., differences in developmental stages), the animal was placed in the test apparatus. As a food reward, a commercial snack brand with peanut flavour was placed in the narrow tube and the 30-minute test session began. At the beginning of each testing session, a small piece of food reward was pushed through the narrow tube to the rats' side of the apparatus by the experimenter to introduce the food reward and motivate the rats. This was repeated by the experimenter every time the animal lost its attention/motivation to the test. In our study, the Collins test was performed until the animals reached the number of 50 paw interventions to get the food reward in 30-minute testing sessions on consecutive testing days.

Pasta matrix reaching test

As a result of the experiences gained from the optimization trials in our study, unlike the original paper, the first two raw spaghetti pieces were dipped in hazelnut cream and presented to the animals to increase the desirability of the spaghetti reward (notably, the rats in our study did not show great interest in spaghetti as such). Before each session, the experimenter also offered a small piece of hazelnut cream to the rat as an introductory step after the animal was placed in the apparatus without the middle gray layer. The test was performed until the animals reached the number of 50 paw interventions to get the food reward in 15-minute sessions on consecutive testing days in our study.

Head-turning asymmetry test

After the test apparatus is adjusted according to the size of the subject (e.g., differences in developmental stages), the animal is placed in the test apparatus. Then, the bottle containing 5% hazelnut syrup water is inserted into the apparatus and the 30-minute test session begins. While placing the bottle containing hazelnut syrup water on the apparatus, a few drops dripping onto the apparatus' floor were perceived as the initial motivation to start drinking.

Special advice

Advice 1

Unlike the original paper (Soyman et al., 2018), we used 5% hazelnut syrup water as a reward during the experiments instead of normal water allowing us to perform this test without any food and/or beverage restrictions. The original study by Soyman et al. (2018) stated that the duration of drinking behaviour was around 180 s per session. In our study, adding hazelnut syrup to the drinking water increased the average drinking behaviour. This increased time spent on drinking behaviour may reduce the overall time (and days) needed for testing in further studies.

Advice 2

Due to the self-occlusion of body parts and significant scale disparities (i.e., animal size close and far from the camera), a single camera location may be unable to record freely moving rats in the depth of the testing apparatus. Therefore, a multi-view approach is highly recommended to capture the freely moving behaviours of rats during the test sessions. We recommend using deep learning-based pose estimation tools such as DeepPoseKit (Graving et al., 2019) and DeepLabCut (Nath et al., 2019) in addition to manual analysis of the head-turning asymmetry analyses for a more objective and precise analysis.

Statistical analysis used in laterality research in animals

The main indexes used to determine paw preference

The strength of the paw preferences of the animals is determined using a laterality index (LI), calculated using the formula $LI = (R\text{-}L)/(R + L)$ (Waters & Denenberg, 1991). According to this equation, R indicates the total number of interactions with the testing apparatus that are conducted with the right paw, while L indicates the total number of interactions with the testing apparatus that are conducted with the left paw. LI has a range from −1.0 (exclusive use of the left paw) to +1.0 (exclusive use of the right paw). A score of 0 indicates that the subject used the left and right paws equally and thus showed no preference. To measure individual lateralization strength independently of the direction bias, absolute LI's are also calculated.

To classify subjects as left-pawed, right-pawed, or ambilateral, the significance of paw preference is determined using the Z-score calculation based on the binomial distribution for each animal $[z = (R-0.5\ N)/\sqrt{(0.25\ N)}]$ (Branson & Rogers, 2006). Animals with a positive Z-score value ($z \geq 1.96$) are scored as R-pawed, whereas those with a negative Z-score value ($z \leq -1.96$) are scored as L-pawed. The other animals are determined to be ambilateral (A). In addition to the laterality index and binomial Z-score, previous research on rodent motor laterality has employed different metrics, called the "RPE" score, which is the number of right paw entries observed in 50 observations, to classify the direction of the paw preferences (Collins, 1968). RPE score can range from 0 to 50, with animals over 25 usually classified as right-pawed (Bulman-Fleming et al., 1997; Collins, 1968), while other cut-off values have been applied (Evenden & Robbins, 1984; Fride et al., 1990; O'Bryant et al., 2011).

While there are some studies in the literature that investigate paw preference in animal studies (Hook & Rogers, 2000; Wells, 2003; Wells & Millsopp, 2009) using the 100 paw response as a sufficient number for statistical analysis, the prevailing trend is to consider 50 responses as an adequate number for many more studies (McDowell et al., 2018; Simon et al., 2022a; Stieger et al., 2022; Wu et al., 2010).

Head-turning asymmetry assessment

Soyman et al. (2018) categorized the rats as right- or left-head-turners if they preferred an aspect equal to or greater than 70% of the total drinking time. The analysis of the head-turning asymmetry test can not only be conducted manually by researchers, but it can also be performed using deep learning-based pose estimation tools such as DeepPoseKit (Graving et al., 2019) and DeepLabCut (Nath et al., 2019).

Discussion

In this article, innovations in methodology, design, and requirements are presented in the context of paw preference in dogs, cats, and rats as a proxy for behavioural lateralization. As of yet, one of the main challenges in the field is that although validated tests to assess paw preference exist for some species, such as rats and mice (Collins, 1968, 1969, 1975), there is no standard test to compare lateralized manual behaviours between individuals, populations, and animal species. One of the main reasons behind the need for a standardized test is that different fine motor abilities and postures must be considered for each species. Given that the study of behavioural lateralization yields crucial insights into animal welfare, laterality research, as well as the clinical neuroscience of lateralization, we aim to overcome these challenges by presenting design requirements and innovations in methodology across species.

We highlight that it is extremely important to consider design requirements for measuring behavioural laterality, such as zoometric fit for different body sizes and developmental ages, test reliability, robustness (structural stability and durability), fast maintenance, and animal welfare, in the design of a new apparatus. Here, we presented six apparatuses to assess paw preference in dogs, cats, and rats.

Both automated and manual FRTs for the dogs were designed with a zoometric fit, allowing for adjustability to accommodate various sizes of dogs while assessing paw preferences. Hence, dogs of any breed and age are able to be tested, irrespective of their physical dimensions. Since the primary goal of the experiment is to ascertain the paw preferences of dogs, the design of FRTs should actively encourage the use of paws rather than reliance on heads or tongues. A pivotal element in this design is ensuring the vertical adjustability of the slot where the animal inserts its paw, thus promoting consistent positioning for each food drop, a crucial factor for the reliability of the research. Considering the inherent diversity in paw lengths among dogs and the individual motivations underlying their behaviour, it becomes evident that the test design should incorporate varying difficulty levels. This strategic adjustment is essential to accommodate the inherent differences in paw lengths and motivational factors among the canine participants, thereby enhancing the overall adaptability and precision of the study. Due to this rationale, both FRTs incorporate a sliding mechanism that can push the food reward over five distinct levels. This sliding mechanism could be operated with a remote control in the automated FRT, while it requires physical manipulation by the experimenter in the manual FRT. The manual FRT, which has the same working mechanism as the automatic FRT, is designed to prevent any situations, such as the automated sliding mechanism malfunctioning and/or the animal being frightened by its sound. Additionally, prior to finalizing the FRTs, prototypes constructed from cardboard material were utilized to conduct preliminary trials involving dogs. Based on the outcomes of these trials, it was determined that modifying the adjustable paw reach range using a vertical mechanism would be more suitable than employing a horizontal mechanism for dogs.

Although the Kong Test is commonly used as the predominant approach for assessing paw preference in dogs (Batt et al., 2009; Marshall-Pescini et al., 2013; Salgirli Demirbas et al., 2019; Simon et al., 2022a; Siniscalchi et al., 2016), there have been debates regarding the reliability of this test due to the tendency of dogs to use their paws in a random manner to stabilize the Kong toy (Wells, 2021; Wells et al., 2016). Another study by Simon, Frasnelli (2013) which examined the relationship between performance in the Kong test and in locomotion tests to determine paw preference in dogs, also reported factors such as left-hemispheric specialization for feeding contexts and unwanted learning effects that may influence the Kong™ Test, leading to potential limitations. Despite the various sizes available and the practicality of the Kong toy in terms of preparation, cleaning, and portability, it is worth noting that the asymmetrical design of the toy, with one hole being larger than the other, may pose a disadvantage. This design feature often leads dogs to primarily focus on accessing the food through the larger hole, which can limit the test's accuracy. Additionally, the mobility of the Kong toy can also contribute to its downsides. Moreover, Plueckhahn et al. (2016) stated that some dogs with smaller sizes exhibited a lack of interaction with the Kong toy, resulting in non-responsiveness. In our previous study, which used the KT and presented the FRT in this study as motor laterality tests (Salgirli Demirbas et al., 2023), we observed that there was a higher percentage of dogs, especially working dogs, interested in the FRT compared to the KT, even though it was not a non-statistically significant trend. The superior performance of the FRT test compared to KT in working dogs may be explained by the fact that working dogs are often trained with a ball and like digging behaviour (Jones et al., 2004). Instead of manipulating the Kong toy, working dogs mostly carried it in their mouths. Furthermore, McGreevy et al. (2010) also reported that some breeds with shorter muzzles achieved a sufficient number of paw responses on the Kong toy test in a shorter time period than the ones with longer noses. Given all the aforementioned factors, it becomes evident that the development of a standardized motor laterality test holds great importance for minimizing discrepancies between dog breeds and individuals. Furthermore, FRTs demonstrate superiority over other invasive techniques, such as the removal of tape from the nose/eye (Quaranta et al., 2004; Tan & Caliskan, 1987) or removal the blanket from the head (Wells, 2003) as well as other methods documented in the existing literature that may be susceptible to learning effects, like paw-shaking (Wells, 2003) and the first stepping test (Tomkins et al., 2010).

Last but not least, the transportability of FRTs is an essential factor in acquiring precise data on dog species, as their paw preferences can be influenced by acute stress exposure (Salgirli Demirbas et al., 2023). Thus, the animals can be tested in the home environment they naturally inhabit, avoiding any external stressors such as novel environments. Other advantages of FRT tests are that there is no adaptation period required for the testing apparatus, and there is no need for food/drink restrictions, even for short durations.

All of the cat paw preference tests presented in this study were designed with a zoometric fit, which are compatible with the majority sizes of cat breeds, with the exception of notably distinct ones like the Maine Coon. Consequently, no modifications to the apparatuses are required. In the first two tests, the combination of cats' excellent sense of smell and the design of the holes in the cups helped cats detect food rewards. Consequently, the test mechanisms' appeal was enhanced through the use of olfactory stimuli in addition to visual cues provided by plexiglass material, thereby increasing its overall attractiveness. The first two tests can also be used as problem-solving tests as well as to determine the paw preferences of the cats. In our previous investigation, we demonstrated that cats require a significantly greater number of paw manipulations to successfully solve Test 2 (Complex Lid Opening Test) compared to Test 1 (Easy Lid Opening Test), indicating that test 2 is more complex than test 1, which is consistent with our design purposes (Isparta et al., 2020). Thus, these setups also allow researchers to test the Task Complexity Theory suggested by Fagot and Vauclair (1991), supported in many species, such as De Brazza's monkeys (Schweitzer et al., 2007) and capuchin monkeys (Lilak & Phillips, 2008). Test 2 assesses two different motor actions simultaneously by requiring cats to open lids and reach for food, which involves distinct motor skills. It is sufficient to place the food reward in the testing apparatus once before the session, as most cats demonstrate enough paw usage without reinserting the reward. Another novel test allows cats to reach food effortlessly by extending their paws, providing a practical alternative for determining paw preference. All three tests require no training or acclimation for cats. They are compact, lightweight, and portable, making it easy to test cats in stress-free environments like their homes. In contrast to some previous investigations where cats were confined within testing apparatuses such as the WGTA, an adapted iteration of the WGTA, or a special cage (Forward et al., 1962; Olmstead & Villablanca, 1979; Yetkin, 2002), our test apparatuses allow cats to freely move around the apparatus, thereby eliminating any physical constraints. While it is widely acknowledged that cats possess distinct individual personalities and exhibit varied responses to different degrees and types of danger or threat circumstances (Carlstead et al., 1993), it is well-established that they tend to employ the "fight-or-flight response" as an active coping strategy (Steimer, 2002). Accordingly, the utilization of WGTA as well as the testing protocols fixing the cat with a neck collar while reaching the moving spotlight implemented by Lorincz and Fabre-Thorpe (1996) are not appropriate methods for animal welfare as they suppress their active coping strategies. Here, another animal welfare issue that should be considered is that a moving spotlight can lead to frustration by causing incomplete hunting behaviour in cats. The "Frustration System", which is triggered when the animals fail to satisfy expectations, be able to obtain resources, or experience a loss of control, may cause behavioural problems such as aggression in subsequent periods (Heath, 2022). Overall, our testing setups and protocols address these concerns and have greater advantages over these methods. In a manner akin to our FRT applications in dogs, these tests also do not require an adaptation period during their implementations; there is no need for food and/or drink restrictions, even for a short time.

One great benefit of the newly designed apparatus for rats is that three different laterality tests are combined, and this significantly reduces the animal's habituation period to the testing apparatus. Under favour of the zoometric fit, the apparatus is adjustable for animal sizes and enables comparable conditions for even different developmental ages. The height of the middle layer in the apparatus is vertically adjustable for Collins Paw Preference Test and Head-turning Asymmetry Test. The compatibility of the Pasta Matrix Reaching Test for all animal sizes can be easily achieved by removing the middle layer from the apparatus since the pasta unit is reachable for all age groups. Moreover, considering to prevent any movement restriction-related stress exposure to the animals during the paw preference determination test, the test apparatus was designed to allow the rats to roam freely in it. Given the diminutive dimensions of rats' paws and their rapid motions, the vital importance of providing easy observation becomes apparent. Therefore, the entire equipment, except the middle layer, is made of transparent plexiglass material. The Collins test involves an interactive procedure between the experimenter and the rat, where a food reward is positioned in the small narrow tube that is precisely equidistant from the left and right walls of the front wall of the chamber. Ease of observation also plays a role in facilitating this process. Only the middle layer was constructed using non-transparent material in order to minimize potential distractions for the animals when they direct their gaze below.

While some of the existing studies that used food-reaching tests did not administer food deprivation before the experiments (Cunha et al., 2017), most of them usually rely on depriving the animal of food or water for motivational purposes (Ecevitoglu et al., 2020; Güven et al., 2003; Tang & Verstynen, 2002), potentially reducing their welfare. Here, the presented innovations in methodology and design increase the animals' motivation without the need for any deprivation. In the capacity of the experimenter, it is worth noting that most of the rats entered the test apparatus themselves when the lid of the apparatus was opened, without the need to place them in the test apparatus. For the experimenter, the presented maintenance considerations and the enhanced observation possibilities will certainly facilitate and accelerate the assessment. The included considerations on research reliability and validity will lead to more conclusive results. It's crucial to consider that some rats might not receive 50 paw responses in a single session because of individual differences. One may assume that rats require more testing sessions in comparison to cats and dogs. In the doctoral dissertation authored by Isparta (2023), the animals were subjected to consecutive testing sessions until the desired number was achieved. Considering that rats are neophobic creatures (Würbel et al., 2017), unlike cat and dog tests, it is recommended that rats be habituated to the test apparatus as described in detail in the procedure section.

All of the testing apparatuses are constructed using transparent plexiglass material, either partially or completely. This choice of material facilitates ease of cleaning and ensures the absence of any potentially harmful substances and/or microorganisms that could compromise the health of animals involved in the testing process, considering animal welfare. In addition to addressing maintenance concerns, using transparent plexiglass material also offers enormous benefits regarding research reliability and robustness considerations. The transparent plexiglass material helps the animals see the food easily, increasing the attractiveness of the tests and the animals' motivation. In addition, this particular feature enables the experimenter's ability to observe the animals' paw movements both during the testing phase and during the analysis of recorded videos. Furthermore, the utilization of plexiglass material contributes to the structural stability and durability of the testing devices.

The precise application for employing the test instrument is equally significant as its proper design, considering animal welfare. As the test procedures are described in detail above, in order to enhance the animals' motivation for the test and eliminate any potential frustration, a piece of food reward was provided to the animals by the experimenters both prior to and following all test sessions.

Our previous studies, applying the presented test apparatuses (Isparta, 2023; Isparta et al., 2020; Salgirli Demirbas et al., 2023), have demonstrated that the percentages of lateralized and non-lateralized animals align with the general range of data obtained in large-scale meta-analyses studies of these species (Manns et al., 2021; Ocklenburg et al., 2019).

The present paper focused on dogs, cats, and rats as they have been the most commonly studied in the field of laterality research. Regardless of the animal species, paw preference determination tests must be easy for the animal to solve, easy for the researcher to apply, and maximally standardized. It is crucial to establish certain design requirements for paw preference studies that align with the biological needs of the species under investigation. The presented innovations in methodology and design increase the animals' motivation without any restrictions from fulfilling their biological and physiological needs. The presented novel testing apparatuses provide simple, practical, and cost-effective methods for the experimenter. The included considerations on research reliability and validity will lead to more conclusive results while providing reproducibility.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Ethical statement

The cat-related section of this paper received ethical approval from the Animal Experiments Local Ethics Committee at Ankara University (2018-17-108), while the dog-related part obtained approval from the same committee (2018-4-39). Prior to the study, informed consent was obtained from the owners of both dogs and cats. All procedures have been conducted in accordance with the ARRIVE guidelines. The rat-related section of this paper received ethical approval from the animal ethics committee of the Landesamt für Natur, Umwelt und Verbraucherschutz (LANUV) in the state of North Rhine-Westfalia (81-02.04.2019.A361). All rat experiments were conducted in accordance with the guidelines specified by Germany's Animal Welfare Act.

Data availability statement

This is a technical paper, and no new data is reported, therefore a data availability statement is not applicable.

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