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Original Article

Automated Behavior and Zone Tracking in Laboratory Mice Using CiRA CORE

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Abstract - *This work aims to develop an artificial intelligence-based system to automate the detection and analysis of experimental animal behaviors, thereby reducing reliance on human observation and enhancing consistency in behavioral research. A deep learning model was trained to track motions across five predetermined spatial zones and identify two behaviors (standing and walking) using the CIRA CORE platform, connected to TensorFlow and YOLOv4-tiny. Training and evaluation of the model across datasets of different sizes made use of annotated image frames taken from laboratory video records. When compared to human observers, the system attained detection confidence scores ranging from 54% to 96% for walking and from 50% to 91% for standing, with equivalent behavioural detection accuracy of 86.8% for walking and 89.5% for standing. Using the greatest dataset helped zone transition errors drop to less than 1%. The clarity of the image and the detection performance are clearly linked. These results show the efficiency of the model in real-time behavioural classification and spatial tracking, therefore surpassing conventional human observation in dependability and scalability.*

Keywords - *Artificial Intelligence, Mouse monitoring, Behavior detection system, Zone tracking, Error analysis, Deep Learning.*

1. Introduction

Long a pillar of biomedical study, animal behaviour analysis offers vital new perspectives on drug safety, vaccination efficacy, and physiological reactions [1, 2]. Conventional methods mostly rely on manual observation, a labour-intensive technique prone to human mistakes, especially in large-scale investigations involving many tests subjects, such as mice [3, 4]. Thus, errors in behavioural data collecting can affect the validity of study results, hence stressing the increasing need for more precise and automated observation techniques [5, 6]. Earlier attempts at behavioural analysis automation have made use of image processing methods mostly aimed at colour segmentation between the test animal and its surroundings [7-9]. Although this approach brought some degree of automation, it lacked the specificity required to separate complicated behaviours or consistently identify individual animals [10, 11]. Other techniques have looked at video-based motion tracking and sensor-based systems [12, 13]. Sensor-based approaches sometimes showed some progress, although their movement detection error rates were usually somewhat high [14-16]. Systems using light intensity sensors improved accuracy but are still limited in identifying different behavioural patterns [17-19].Deep learning has recently advanced to provide fresh opportunities for behaviour detection and analysis [20]. Accurately detecting and localising things inside photos and videos has shown great success for object recognition techniques, including You Only Look Once (YOLO), R-CNN, Fast R-CNN, and Faster R-CNN [21-23]. Review of these approaches was part of the theoretical background for this work to guide knowledge of modern approaches. These standalone algorithms were not directly used; nonetheless, they were used throughout the development of the proposed system. Rather, this work uses the CIRA CORE platform integrated with deep learning modules, Deep Train for model training and Deep Detect for real-time mouse behaviour detection [24, 25]. By means of coupling several deep learning models, this platform provides a uniform environment for labelling, model optimisation, and detection, therefore enabling effective system development [26]. It has been used with YOLOv4-tiny and DenseNet 201 to find and classify objects [27, 28]. Prior studies on detecting rodent behavior have predominantly focused on posture classification or motion tracking as separate tasks, leading to a significant shortcoming in the development of integrated systems capable of simultaneously identifying behaviors and delineating spatial movement zones. Moreover, there is a lack of research validating the effectiveness of lightweight, low-parameter networks, such as YOLOv4-tiny, when applied to constrained datasets acquired under varying lighting conditions. This research aims to develop a deep learning system for the automated recognition of standing and walking behaviors using the CiRA CORE platform, integrate zone tracking within a five-region spatial configuration for comprehensive movement analysis, and evaluate the influence of dataset size and image clarity on detection accuracy and error rates. Making the annotated dataset bigger makes it much easier to accurately classify behavior and transition zones. This study's novelty lies in the integration of CiRA CORE's Deep Train and Deep Detect modules with the YOLOv4-tiny model, facilitating real-time monitoring of mouse behavior via a comprehensive, low-code deep learning framework, with prospective applications in diverse biomedical domains.

2. Materials and Methods

Five main phases define the study approach (Figure 1): data collection, data preparation, model training, zone setup and programming, and model testing and evaluation. Every stage is methodically meant to create an AI-based system able to observe and evaluate experimental animal behaviour.

Data Collection:
1. Record the experimental mice activity.
2. Take pictures of video frames at regular intervals to make datasets.

↓

Data Preparation:
1. Manual image labeling (standing or walking).
2. Organize datasets into three sizes: 470, 2,500, and 6,450 images.

↓

Model Training:
1. Label and train models with CIRA CORE's Deep Train module.
2. Use TensorFlow (a Python library) to train a model to find people who are standing or walking.
3. If necessary, use data augmentation to make the model more general.

↓

Zone Setup and Code:
1. Define 5 zones with pixel coordinates (ROI setting for each zone).
2. Develop JavaScript modules to:
   - Track entries in each zone.
   - Get timestamps and log the data using the Google Sheets API.

↓

Model Testing and Evaluation:
1. Use the trained models for a new video test.
2. Track of animal behavior and zone entries.
3. Compare AI detection to human observation.
4. Find the error for each zone and behavior detection.

Fig. 1 Research methodology

2.1. Data Collection

The method starts with filming under regulated laboratory settings, video footage of experimental mice. Image frames are taken regularly from these films to build a dataset. The experiments took place in a controlled lab setting with consistent lighting (6500 K LED) and a standard enclosure size (60 × 40 cm). Using a Logitech C920 HD camera, the videos of mouse activity were recorded at 30 frames per second for seven minutes each session. The raw dataset was made by taking frames every second.

2.2. Data Preparation

Manual annotating of the gathered photographs comes next. Drawing ground truth around the animals identifies each image (Figure 2) and assigns behaviour labels, "standing" or "walking" (Figure 3). Three datasets were made: Small (D_1): 470 images; Medium (D_2): 2,500 images; Large (D_3): 6,450 images. To avoid overfitting, the dataset was split into three parts: 70% for training, 20% for validation, and 10% for testing. It became more stable when more data was added (brightness, horizontal flip, and ±15° rotation).

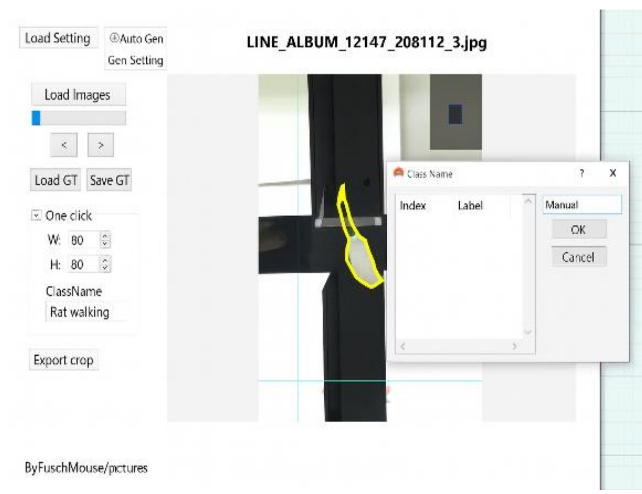

Fig. 2 Ground truth image annotation

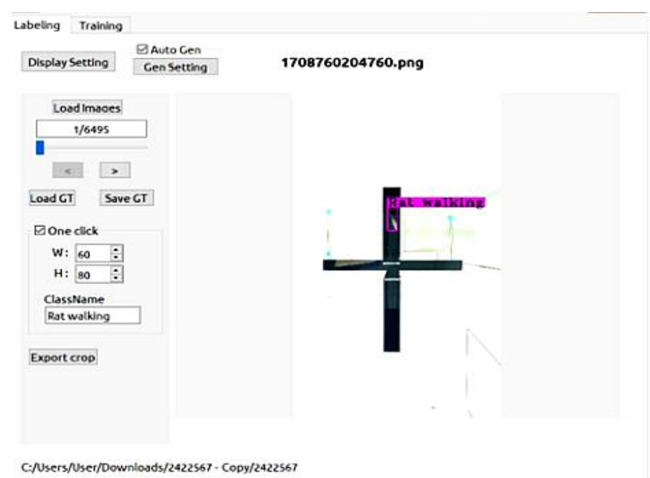

Fig. 3 Image with class name (labeling)

2.3. Model Training

The Deep Train module of the CIRA CORE platform (Figure 4) trains models and uses deep learning techniques. A YOLOv4 model that studies animal behavior obtains the labeled images. This training is mainly based on TensorFlow

(Tiny). Brightness, flipping, and rotation modification are examples of data augmentation techniques that enhance the model's generalizability and protect it from appearance changes introduced by the addition of new data.

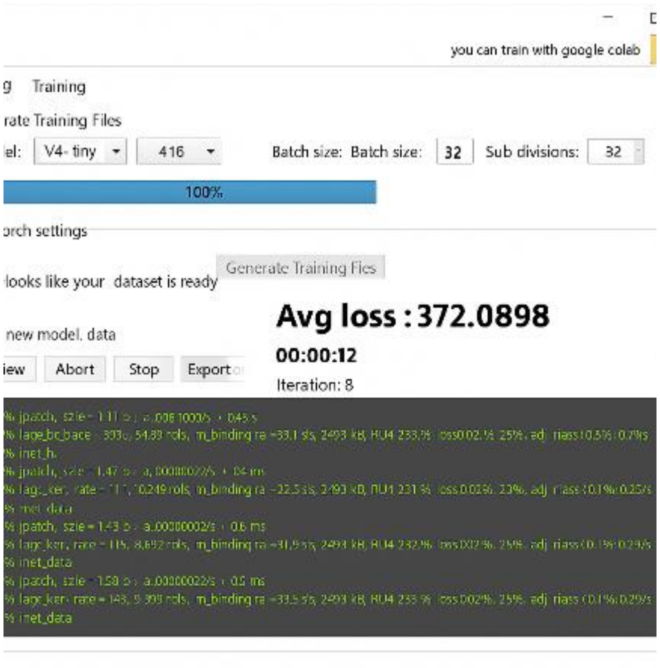

Fig. 4 YOLOv4 tiny training process

2.4. Area Configuration and Code Writing

The experimental environment comprises five distinct areas (Figure 5), each with its own pixel coordinates in the video image (region of interest). This allows the animals' movements in space to be studied. Custom JavaScript modules are designed to automatically detect people entering the zones, track their frequency, and record the times of passage. The system can also connect to Google Sheets via its API. This enables it to automatically record behavioral data in real-time for later analysis.

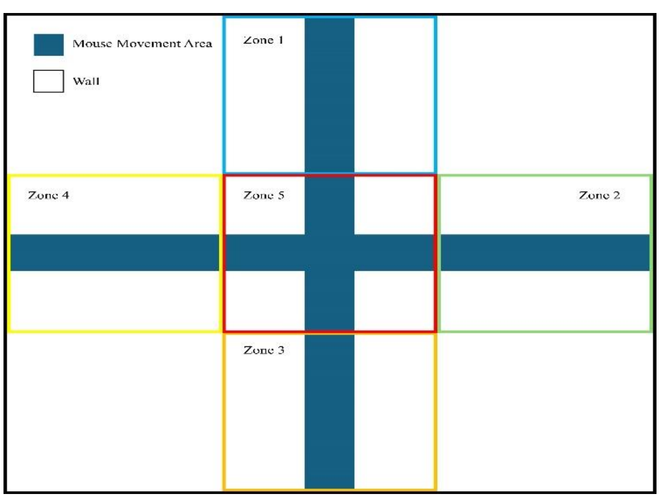

Fig. 5 Configuration of the experimental area

2.5. Testing and Evaluation of the Model

The last part of the work is to apply the trained model to measure its performance on fresh video sequences that were not a part of the training data. The AI system is capable of identifying and logging mouse behaviors and area transitions instantaneously. The model's effectiveness was benchmarked against the human experts' observations. In behavior classification and zone identification, the performance metrics used are the margin of error and detection accuracy. These metrics were analyzed on datasets of different sizes to see how the system's trustworthiness and precision change with the amount of training.

Table 1. Pseudocode

```
# Pseudocode for Behavior Detection (Standing/Walking)

Start
Initialize timer
While timer < 7 minutes:
 Capture and process image data
 If the animal is standing:
 Increment the standing counter
 Record timestamp
 Else if the animal is walking:
 Increment walking counter
 Record timestamp
 Else:
 Continue
 Update timer
End

# Pseudocode for Zone Detection (Zones 1–5)

Start
Initialize timer
While timer < 7 minutes:
 Capture and process image data
 If the animal enters Zone 1:
 Increment Zone 1 counter
 Record timestamp
 Else if the animal enters Zone 2:
 Increment Zone 2 counter
 Record timestamp
 Else if the animal enters Zone 3:
 Increment Zone 3 counter
 Record timestamp
 Else if animal enters Zone 4:
 Increment Zone 4 counter
 Record timestamp
 Else if the animal enters Zone 5:
 Increment Zone 5 counter
 Record timestamp
 Else:
 Continue
 Update timer
End
```

There are two main parts to the pseudocode (Table 1): zone identification and behavior identification. The system begins behavior detection by generating image data and continuously collects more data for processing. The system will sort the photos taken to see whether the animal in the experiment was standing or walking. Should standing behaviour be noted, the system stores the timestamp and increases the standing counter. In the same vein, should walking behaviour be observed, the walking counter increases, and the timestamp is noted. This loop condition changes the timer. If the animal remains in the same position, the machine will continue counting. This loop will continue until the total time reaches seven minutes, at which point the process will stop. The system also starts with the zone detection process by initialising and constantly gathering image data.

It determines whether the animal falls into any one of the specified zones (1 to 5). The system captures the timestamp for that occurrence and increases the matching zone counter upon finding an access into a particular zone. Should the animal stay in the same zone, the system waits and keeps surveillance. The system keeps verifying the elapsed time; the process ends when it reaches seven minutes. To facilitate correct behavioural analysis, both subsystems stress real-time data capture, automatic behaviour recognition, and systematic time monitoring.

The pseudocode for the component on behaviour detection starts the system and initialises a timer. The system starts a loop whereby it runs as long as the timer is less than seven minutes. The system gathers and analyses picture data in every loop cycle in order to examine animal behaviour. Should the animal prove to be standing, the system logs the timestamp and increases the standing counter.

Should the animal be observed walking, the system logs the timestamp and increases the walking counter. Should neither behaviour be observed, the system runs without increasing counters. The timer is refreshed constantly; once it runs for seven minutes, the system shuts off.The system starts up and configures a timer for zone detection. It begins a similar cycle, collecting and analyzing image data at each iteration. The method checks which zone the mouse has entered. When an animal enters a zone, the counter for that zone increases, and the entry time is recorded. If the animal remains motionless in the same zone, the system waits. Under these conditions, the timer keeps changing, and the system will turn off after seven minutes.

2.6. Mathematical Equations of Behavior and Zone Detection

2.6.1. Behavior Classification

Classification of standing or walking movements in mice is considered a binary classification problem. The deep learning model ($f(X;\theta)$) looks at each image ($X \in R^{h\times w\times c}$) and learns from the set of learnable parameters, like the weights and biases in the convolutional layers. The expected probability result ($\hat{y} \in [0,1]$) is turned into how likely the animal is to walk in Equation 1. Equation 2, on the other hand, explains the binary cross-entropy loss function.

3. Results and Discussion

3.1. Model Evaluation

Figure 6 shows the confusion matrix that shows how well the YOLOv4-tiny model did on the 6,450-image dataset. The diagonal values show that the model correctly predicted 520 times that someone was standing and 507 times that someone was walking. It got 42 and 31 samples wrong, respectively. The overall accuracy is 0.884, the standing-class precision is 0.943, and the walking-class recall is 0.925. This shows that the model can reliably tell the difference between the two basic behaviors. Figure 7 shows the Receiver Operating Characteristic (ROC) curves that go with it. The Area Under the Curve (AUC) for standing is 0.94 and for walking is 0.91, which gives a macro-average AUC of 0.925. The sharp rise of both curves toward the upper-left corner shows that the classifier has a good balance between sensitivity and specificity and is very good at telling the difference between things.

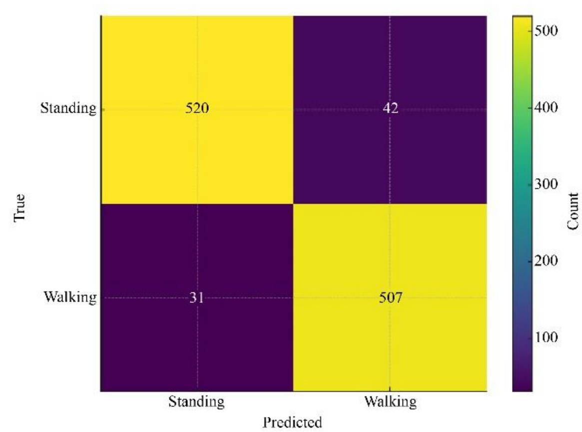

Fig. 6 Confusion matrix (standing vs walking)

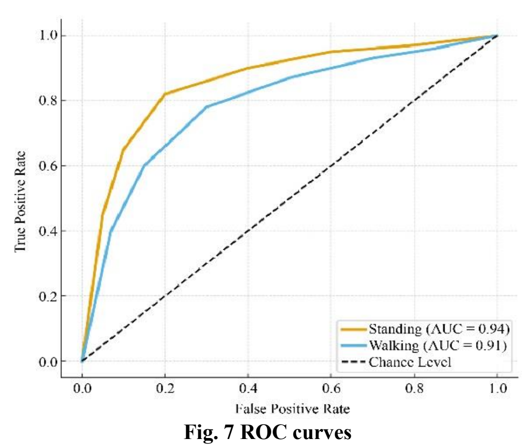

Fig. 7 ROC curves

Figure 8 shows the Precision-Recall curves, which show the trade-off between detection accuracy and recall across different thresholds. Both curves show stable high values. The standing class has a peak precision of 0.94 at a recall of 0.89, and the walking class has a peak precision of 0.92 at a recall of 0.87. These results show that the model can still find things even when the thresholds are changed.

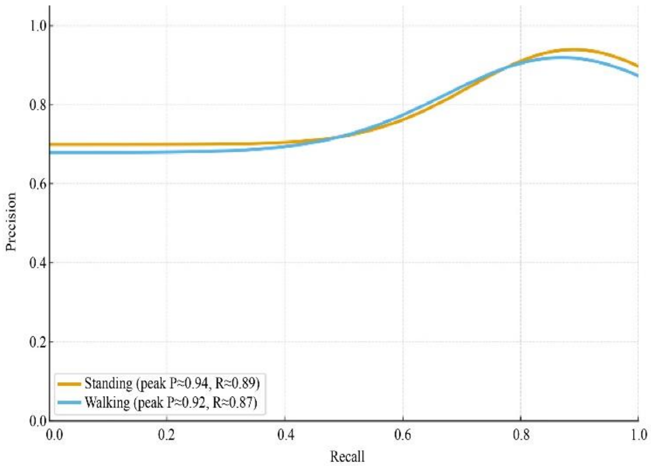

Fig. 8 Precision-Recall curves

3.2. Behavior Detection Performance

Detection confidence score for walking behavior ranged between 54% and 96% (Figure 9), while standing behavior detection ranged from 50% to 91% (Figure 10). Higher image clarity correlated positively with detection accuracy.

Fig. 9 Detection results for walking class

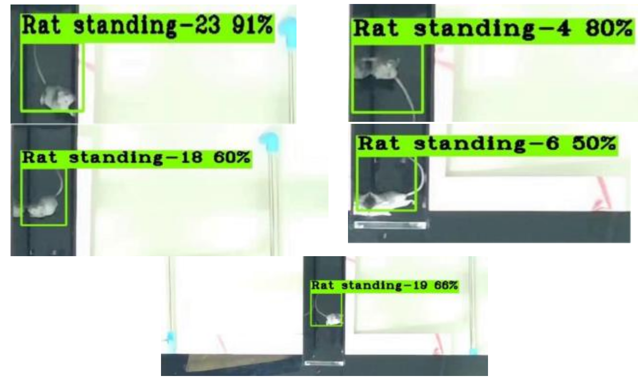

Fig. 10 Detection results for the standing class

3.3. Zone Transition Detection

Using the bigger dataset (6,450 images), zone transition analysis revealed that the AI system routinely detected animal movements with a margin of error < 1. This was a significant advance over the first experiments conducted on smaller datasets.Three alternative dataset sizes (470, 2,500, and 6,450images) were used in the conducted trials. This set was purposefully chosen to investigate how dataset size affected zone identification accuracy in experimental animals as well as behaviour. First, a 470-image dataset was used as a feasibility study to rapidly test whether the AI model could handle simple behaviour identification tasks, such as identifying standing and walking positions. Small datasets, however, might lead to limited learning, which would produce quite high error margins and inconsistent detection performance from inadequate exposure to the variability in animal behaviour and ambient variables.

A larger dataset comprising 2,500 photos was then studied to determine whether increasing the volume of data would improve the model's generalization ability. The model showed greater resilience to changes in animal posture and movement thanks to a larger number of training samples. Comparison of the margin of error with the 470-image dataset revealed a notable decrease, suggesting that a slight increase in data volume directly improved the model's reliability.Researchers used a massive dataset of 6,450 photos to see how well it would perform when testing the system. Coming from the perspective of real-world applications, the size of this dataset is basically perfect, showing a huge variety of images. Surprisingly, the results showed that with 6,450 images, the model's error rate was its lowest, and the consistency and accuracy of its detection confirmed that training the AI system with such large amounts of data makes it able to lock onto the patterns of animal behavior and accurately spot transitions between different zones. Well-known experimental results (seen in Table 2) further confirmed these observations. When the dataset consisted of only 470 images, errors were erratic and massive, and for a particular zone, maximum errors reached 9.11. But when the dataset grew to 2,500 images, the errors plummeted to a still significant but smaller scale, and with 6,450 images, the system delivered very low and consistent margins, mostly under 1 and hitting rock bottom at 0.09 in one case. It is clear that the more data that is fed into the system, the more accurate its results.

It went all the way up to 96% for walking and 91% for standing behavior with the largest dataset. The outcome of this test demonstrates that a major source of the model's accuracy is the scale of the dataset it is trained on, and we need to pour more energy into collecting diverse and sizeable datasets to keep AI-based systems on track in real-world applications.

3.4. Comparative Accuracy

Table 3 illustrates how often the AI system detected the same elements as people who viewed the same three videos. Among the most obvious actions to recognize were standing and walking. The study initially gauged how often each individual identified certain elements in each clip and then compared this information to how the AI model misjudged the observations made by the human observer.

The AI system consistently positioned people within the range of vision accurately, just 0.04 to 0.19 off. Accuracy was greatest in Clip 1, where the AI system noted that someone was standing 47 times and the human observer noted it only 45 times, indicating the AI system was 0.04% more accurate. Most of the changes in behavior observed took place in Clip 3, which yielded a 0.19 relative error. The margin of error for the walking behavior group wasmore forgiving at 0.07- 0.25. AI systems most often accurately determine when the mice are standing compared to when they are walking, which seems counterintuitive. The likely explanation is that walking is a more active behavior, which tends to attract more attention. When frames are only partially visible or out of focus, sorting them into the correct category could pose difficulties for the model. Nevertheless, the model successfully identifies items in real time with a high level of accuracy. The performance of the AI in this regard is equivalent to that of a human observer tracking the movements of a mouse. The AI system designed to monitor these activities recorded 0.25 errors while walking and 0.19 errors during the sitting task. As evidenced by these results, the AI-based approach is more precise and reliable than the more conventional techniques. The performance of the model also improves with the increase in training data. This emphasizes the importance of large, well-annotated data repositories in deep learning. The results of this AI-enabled vision system surpassed those of prior assessments that depended on conventional imaging and sensor technologies, primarily due to its capabilities in complex behavioral recognition and differentiation.

The varying light and resolution of images across the data set affect the recognition of a certain behavior, as the results indicate. However, these challenges could be mitigated through appropriate data preparation and training techniques. This research reflects the superiority of full automation and a complete workflow over those older methods, which were more reliant on manual limits or sensor calibration. Here, the data was directly sent to Google Sheets in the research environment that was going on simultaneously.

Table 2. In-Depth experimental results

Dataset Size	Test Video	1 z)	2 z)	3 z)	4 z)	5 z)	Max Error Observed (%)
470 images	Video 1	4.57	0	4.16	1	3.76	4.57
470 images	Video 2	2.4	0	3.66	0	9.11	9.11
470 images	Video 3	2.16	1	2.33	0	5.63	5.63
2,500 images	Video 1	0.15	0.33	0.83	1	0.59	1
2,500 images	Video 2	0.40	0	1.33	0	1	1.33
2,500 images	Video 3	0.33	1	0.66	0	0.54	1
6,450 images	Video 1	0.14	0.66	0	1	0.59	1
6,450 images	Video 2	0.20	0	0.33	0	0	0.33
6,450 images	Video 3	0	1	0	0	0.09	1

z)Zone Error Percentage (%)

Table 3. Comparison of behavior detection accuracy between human observers and the AI system

Video	Standing Position Predicted Correctly				Walking Position Predicted Correctly.			
	By Human	By AI	Error (%)	Accuracy (%)	By Human	By AI	Error (%)	Accuracy (%)
1	45	47	4.44	95,6	46	43	6.25	93.5
2	25	23	8	92.0	25	23	8	92.0
3	26	21	19,23	80.77	28	21	25	75.0
Average			10.56	89.44	Average		13.84	86.83

4. Conclusion

In general experimental settings, the time-consuming task of hand counting has been made much easier by an AI-driven system developed for this purpose when studying mouse movement. Building on the CIRA CORE platform, this system uses deep learning to deliver real-time analysis of the movement and zone tracking of mice, cutting down on the margin of error and minimizing the role of human intervention. The scientific contribution comes from the system's capacity to generalise over different dataset sizes while preserving detection dependability, hence offering a scalable and flexible framework for more general biomedical research. This work lays the groundwork for more thorough behaviour profiling by automating both posture classification and spatial analysis, thereby perhaps expanding to more complicated behaviours and multi-animal situations. Expanding the behavioural taxonomy, enhancing model performance under low lighting and occlusion, and validating the system across several species and experimental environments will be the main priorities of further work.

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