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Research report

# Interactive virtual objects attract attention and induce exploratory behaviours in rats

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ABSTRACT

Animals use visual information to recognize the value of objects and respond with different behaviours, such as evasion or approach. While rodents show defensive behaviour toward an artificial looming stimulus that mimics an approaching avian predator, the visual feature that attracts them to targets with positive value, such as prey, remains unclear. Here, we reveal that rats show curiosity-related behaviours towards a virtual object on screen when it moves interactively with their movements, whereas they show less response to a static object, a regularly moving object, or interactive dislocation of the background. To mimic evading prey, we programmed the object to shrink when touched. Rats preferentially responded to interactive shrinking over interactive enlargement. These results suggest that rats exhibit a selective response to interactive objects. This would seem to be an efficient strategy for finding optimal prey using the evolutionarily conserved prey-predator relationship.

## 1. Introduction

The evolutionary arms race between a predator and its prey is critical for ecological stability [1,2]. A prey must try to evade and/or hide from a visually approaching predator, while a predator must detect hidden prey against background objects. Recent studies with a screen chamber revealed the rodents showed defensive behaviours when they recognized an artificial looming or sweeping stimuli that mimicked an approaching or foraging avian predator [3,4]. This behavioural paradigm was successfully connected to uncover the relevant midbrain visual circuits for escaping and freezing [5,6]. These reports used the artificial stimuli for mimicking a predator to study the defensive behaviours and the underlying neural circuits of the prey, but there has been a hurdle to study hunting behaviours and the underlying circuits of a predator because the visual feature of a prey that attracts the predator with positive value remains unclear. Interestingly, animals' playing or object exploration behaviour has been hypothesized as a training behaviour of the hunting behaviour because of their structurally indistinguishable actions [7,8]. Indeed, mammalian predators tend to play with the object which generally have features of typical prey items, such as small size or rapid escaping movement [9,10], and the animals tend to explore more or interact more with the objects when they feel hunger [11–13]. Based on these reports, recent researches studying hunting behaviour exploited the object interaction behaviour as a hunting-like behaviour, and used a non-prey object as a fake prey [14–16]. Despite of the recent findings, the visual features of the prey that attract the mammalian predator remains unclear due to the lack of the behavioural paradigm. For example, amphibian, a toad prefer a worm-like visual object that moves parallel to their movement direction [17], but we still have no clue which visual features are chased by the rodents. Recent studies have shown that rodents also use visual information to hunt prey [18,19], and in this sense, we made a chamber apparatus with a screen monitor to present a visual object to rats, which are strongly motivated to interact with various inedible items [20,21], to find out the visual features that attract them to explore and interact with.

## 2. Materials and methods

### 2.1. Experimental subject details

Male Iar:Long-Evans rats with an outbred genetic background (SLC,

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Japan) were obtained at 6 weeks of age and housed for 2 weeks before the experiments. All experiments were conducted with 8-week-old rats. Twenty-eight rats were used in total, with four rats used for each of the following experimental paradigms: static, regularly moving, interactively moving, interactive background, interactive target, interactively enlarging, and interactively shrinking. Rats were housed two per cage under a 12 -h light/dark cycle with ad libitum access to food and water. All animal care and experimental procedures were performed in accordance with protocols approved by the directives of the Animal Care and Use Committee of the Korea Advanced Institute of Science and Technology (approval number KA2016-52).

### 2.2. Chamber configuration

The chamber (KAIST Biocore Center) was 60 × 60 × 60 $\text{cm}^3$ in size and was formed primarily of black, non-transparent acrylic walls and floor. One wall was transparent, allowing us to present virtual objects on a 32″ monitor (LG Electronics, South Korea). The chamber was fitted with two webcams (HD Pro Webcam C920; Logitech, USA), one at the top and one at the rear; they were used to record each experiment, and the rat’s location, head angle and screen-touching motions. Each video was obtained as 15 fps and processed through conventional OpenCV (Intel, USA) written in Python (Python Software Foundation, USA).

### 2.3. Object interaction test

All behavioural tests were conducted during the dark cycle. The rats were habituated to the test chamber with an blank monitor (white screen) for 10 min per day for 5 consecutive days. On the 6th day, they were habituated to the chamber with the empty monitor for 10 min, then were presented the virtual object for 5 min (object + session) and then left in the chamber with the blank monitor for an additional 5 min (object- session). Seven different types of virtual objects were presented in the object + sessions, and six to eight rats were used for each object stimulus (static, 7; regularly moving, 6; interactively moving, 7; background moving, 8; target moving, 6; enlarging, 6; shrinking, 6; total $n = 46$).

### 2.4. Virtual object presentation

All virtual objects were created using the Unity 5.1.5 game engine (Unity, USA) and the Unity Scripting API C# software (Microsoft, USA). The utilized static object was a circle that had a diameter of 8 cm and remained stationary (*see Video S2*). The regularly moving object was the same circle undertaking a continuous left-right movement along a span of 9.7 cm at a speed of 13.8 cm/s (*see Video S3*). The interactively moving object was the same circle; when touched by the rat, it moved leftward or rightward to the end point of the span at 13.8 cm/s (*see Video S4*). For the background experiment, a black-and-white checkered background was presented on the monitor along with the virtual object; when touched, the background moved at the distance and speed described above. The enlarging object became enlarged when the rats touched it; it started at the size of the static object and enlarged at an angular speed of 162°/s (*see Video S5*). The shrinking object had the same start size, and shrank at a speed of 162°/s when the rat touched it (*see Video S6*).

### 2.5. Object touching to alter the shape or position of the object

We defined object touching as a rat being so close to the target and touching the object so more than 30% of the total object was hidden from the rear-view camera. When the rat touched the object, the prescribed signal was sent to the Unity code, which triggered alteration of the object’s shape or position.

### 2.6. Video analysis

To extract target body postures of rats, we used DeepLabCut [22], which is a deep-learning-based markerless pose-estimation tool. For each top-view video, we annotated 30 images for the head center, nose and body center. We used ResNet50 [23] as the main Convolutional Neural Network layers. The training fraction, which represents the ratio of training data to the annotated data, was 0.95. The total training epoch (iteration) was 1,030,000 and the batch size was 4. After the network trained, the training error was 0.91 pixels and the test error was 4.2 pixels. After the deep-learning model was trained, all video frames were evaluated to obtain the locations of body center, head center and nose (*see Video S1*).

### 2.7. Behaviour analysis

The total time each rat spent in the interaction zone was determined as the cumulative duration the rat spent with position of its body center within the rectangular 60 × 20 $\text{cm}^2$ area in front of the screen. We divided the chamber with the sub-sectors of 50 by 50, generated a bivariate histogram for each sector, and performed Gaussian smoothing with a standard deviation value of 2 to obtain each heatmap. Representative track tracings were generated by tracing the body center of the rat for 5 s after object touching.

The gazing-like behaviour score was calculated as the ratio of time spent gazing-like behaviour from the non-interaction zone versus the total time spent in the non-interaction zone. A rat was considered to do gazing-like behaviour if its head was angled toward the screen within certain ranges (*see Fig. S1*). The range of the head angle was determined as a possible range of the head angles that the head directions can point on the screen on each section.

Latency to object recognition was calculated as the duration between the initiation of the object + session and the onset of object gazing-like behaviour by the rat.

### 2.8. Statistics

All data analyses were performed using SigmaPlot 12.0 (Systat Software, USA). For parameters that followed a normal distribution (Shapiro-Wilk test, $P > 0.05$), differences between two groups were analyzed with the Student’s *t*-test and comparisons of three or more groups were performed using analysis of variance (ANOVA). Between-session differences in the same rat were analyzed with the paired *t*-test. The Mann-Whitney U test was used for data that were not normally distributed. Equal variance was determined in preliminary tests and Welch corrections were deemed to be unnecessary. The Tukey post-hoc method was used to correct for multiple comparisons in one-way ANOVA, and the Student-Newman-Keuls method was used to perform Kruskal-Wallis one-way ANOVA on ranks. All Student’s *t*-test were two-tailed, and p-values < 0.05 were considered statistically significant. Detailed statistical information, including test names, sample sizes, sample types, exact p-values and statistical values, is provided as an independent file in the supplementary information (see *Statistics Table*).

## 3. Results

We designed a chamber that had a screen monitor at the front, on which we presented a virtual object stimulus, and cameras at the top and rear, which we used to record the rats’ responses (Fig. 1a). We used the DeepLabCut algorithm [22], which is a deep-learning-based markerless detection algorithm, to detect the body center, the head center and the nose of each rat (Fig. 1a, *see Video S1*). The head direction was determined by drawing a line drawn between the head center (midpoint of ears) and the nose, and the head angle was calculated as the angular difference between the head direction and a perpendicular line drawn with respect to the screen surface (Fig. 1a, *see Fig. S1a and Video S1*). All

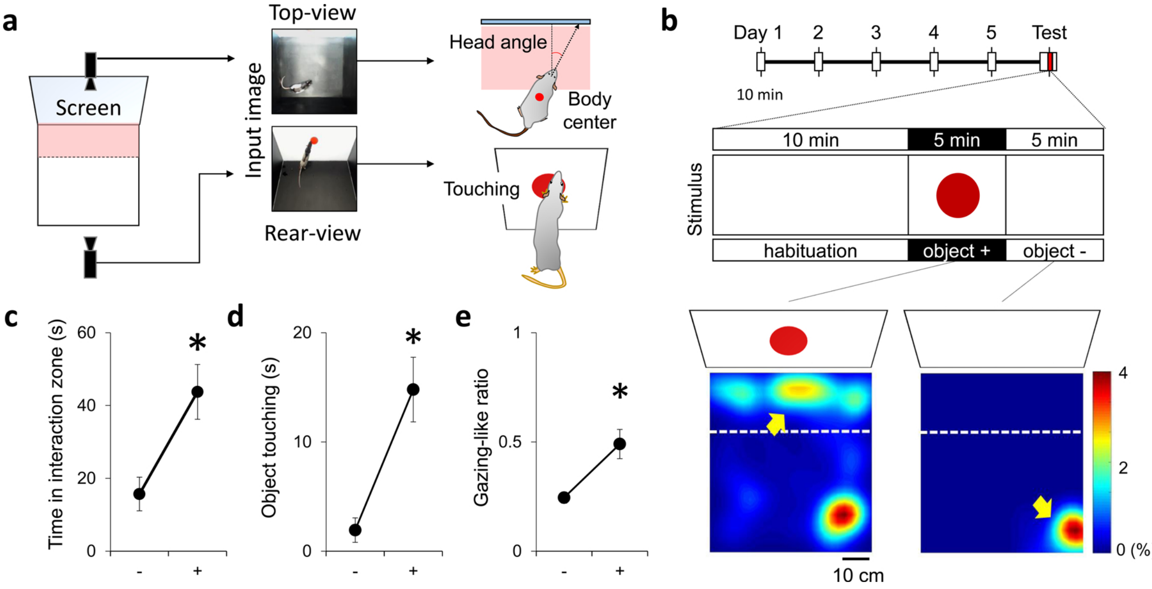

**Fig. 1. Virtual object stimuli elicit active exploration. a.** Schematic figure of the experimental chamber system and paradigm. The pink-shaded area shows the interaction zone. **b.** *Top,* experimental schedule for the object-exploration test. *Bottom,* representative heatmaps showing the time spent in the chamber. *Bottom left,* time spent of the object + session. *Bottom right,* time spent of the object- session. Arrows indicate the area that shows a clear difference between the sessions. The color bar indicates color code of the heatmaps. **c.** Time spent in the interaction zone ($n$ = 7; P = 0.0007). **d.** Object-touching durations ($n$ = 7; P = 0.0009). **e.** Gazing-like ratios obtained ($n$ = 7; P = 0.005). **c–e.** Brighter lines indicate individual data. Circles and error bars represent mean ± SEM.

rats were habituated to the chamber without stimulus (empty screen) for 10 min per day for 5 days (Days 1–5), and then exposed to virtual objects on Day 6 (test day) (Fig. 1b). A test consisted of three sessions in sequence: a 10-minute pre-test without any stimulus (habituation), a 5-minute test with a stimulus (object+), and a 5-minute post-test without any stimulus (object-) (Fig. 1b). We found that rats stayed in the interaction zone for about 3-fold longer during the object + session than the object- session (Fig. 1c; object-, 15.687 ± 4.619 s; object+, 43.756 ± 7.527 s; P = 0.0007). They also spent about 6-fold longer touching the object in the interaction zone during the object + session compared to the object- session (object touching during object- session was measured as touching duration of the empty screen; Fig. 1d; object-, 1.929 ± 1.121 s; object+, 14.793 ± 2.958 s; P = 0.009). To quantify the rats' visual perception and awareness of the virtual objects, we measured the gazing-like ratio, which was calculated as the time spent gazing-like behaviour at the object from outside the interaction zone divided by the total time spent outside the interaction zone. A rat was assumed to do gazing-like behaviour at the object when its head angle was directed toward the object within a certain range (*see Fig. S1b*). We excluded gazing-like behaviour within the interaction zone because the rats often looked up at and/or stood in front of the object to interact with it, making it difficult to measure the head angle. Since there was no virtual object in the object- session, the gazing-like time of the object- session was calculated as the time spent with a head angle within the same range used for the object + session. The gazing-like ratio was significantly higher in the object + session than the object- session (Fig. 1e; object-, 0.245 ± 0.026; object+, 0.490 ± 0.067; P = 0.005) indicating that the rats were more aware of the screen during the object + session. These results show that the virtual object stimulus drew the rats' attention and prompted them to explore.

We hypothesized that the most clear visual feature displayed by prey in a hunting situation was likely to be an escaping movement. Thus, we tested the rats' response to moving objects. Three groups of rats were exposed to a consistent visual object exhibiting three different movements: static (S; Fig. 2a, *see Video S2*), regularly moving (R; Fig. 2b, *see Video S3*), and interactively moving (IT; i.e., movement in response to touch; Fig. 2c, *see Video S4*). The animals actively approached the static, regularly moving and interactively moving objects, as reflected by 3-fold, 2-fold and 3-fold increases, respectively, of the time spent in the interaction zone compared to the no-stimulus condition (object-) (Fig. 2a; object-, 3.137 ± 0.924 s; object+, 8.751 ± 1.505 s; P = 0.007; Fig. 2b; object-, 4.251 ± 1.534 s; object+, 8.301 ± 1.648 s; P = 0.008; Fig. 2c; object-, 5.465 ± 1.779 s; object+, 18.102 ± 1.962 s; P = 0.0009). However, the rats showed significantly higher responses to the interactively moving object, spending about 2-fold longer in the interaction zone, compared to their responses to the static and regularly moving objects (Fig. 2d; S, 43.756 ± 7.527 s; R, 48.422 ± 5.299 s; IT, 90.508 ± 9.808 s; S vs. R, P = 0.690; R vs. IT, P = 0.004; S vs. IT, P = 0.002). They also spent 2-fold longer touching the interactive object than the other objects (Fig. 2e; S, 14.793 ± 2.958 s; R, 18.110 ± 4.104 s; IT, 32.445 ± 3.129 s; S vs. R, P = 0.502; R vs. IT, P = 0.017; S vs. IT, P = 0.004). There was no significant difference in these parameters between the static and regularly moving objects (Fig. 2d, e). Interestingly, the gazing-like ratios were not significantly different among the three groups (Fig. 2f; S, 0.370 ± 0.070; R, 0.339 ± 0.039; IT, 0.479 ± 0.065; P = 0.271), suggesting that all three object types had similar levels of visual salience. To assess whether the rats were better able to initially perceive the regularly moving object because of its dynamic movement, we measured the latency to first object recognition (i.e., that between the onset of stimulus presentation and the first onset of gazing-like behaviour). However, there was no significant difference in the latency to object recognition across all three stimuli (Fig. 2g; S, 6.025 ± 4.457 s; R, 5.678 ± 4.815 s; IT, 9.480 ± 5.847 s; P = 0.659), indicating that the increased times spent in the interaction zone and in touching the object did not reflect variations of visual salience or the rats' perception abilities. During the object + session, the rats did not exhibit significantly different object-touching times across the three object types in the early phase (0–2.5 min) of the session (Fig. 2h; S, 14.355 ± 2.751 s; R, 12.549 ± 3.050 s; IT, 19.080 ± 1.199 s; P = 0.172). In the late phase (2.5-5 min), however, the rats spent significantly longer touching the interactively moving object compared to the other objects (Fig. 2h; S, 0.433 ± 0.433 s; R, 5.011 ± 1.691 s; IT, 11.276 ± 2.224 s; S vs. R, P = 0.067; R vs. IT, P = 0.032; and S vs. IT, P < 0.001). This reflected that the rats exhibited persistent interest in the interactively moving object, whereas their interest in the other objects decreased over time. This might correlate with previous

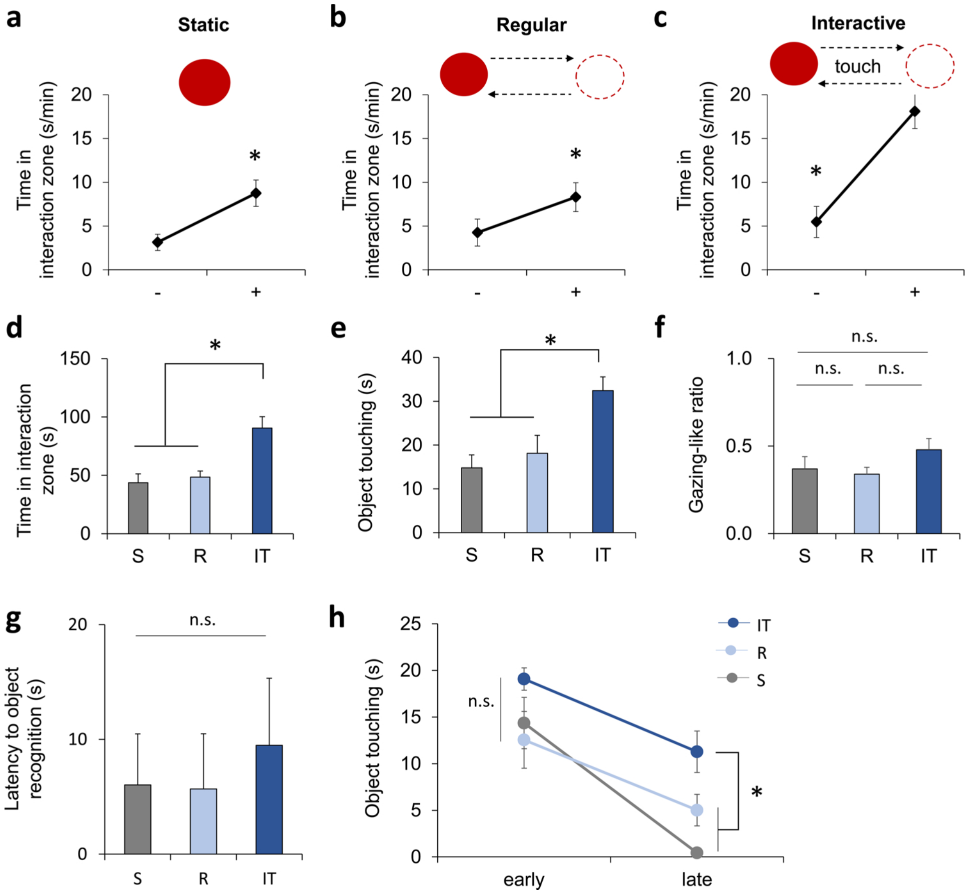

**Fig. 2. Rats show higher reactions to an interactively moving object than a regularly moving one. a.** Time spent in the interaction zone of the static object during the object- and object + sessions (*n* = 7; P = 0.007). **b.** Time spent in the interaction zone of the regularly moving object during the object- and object + sessions (*n* = 7; P = 0.008). **c.** Time spent in the interaction zone of the interactively moving object during the object- and object + sessions (*n* = 7; P = 0.0009). **d.** Time spent in the interaction zone (*n* = 7, 6, 7; S vs. R, P = 0.690; R vs. IT, P = 0.004; S vs. IT, P = 0.002). **e.** Object-touching duration (*n* = 7, 6, 7; S vs. R, P = 0.502; R vs. IT, P = 0.017; S vs. IT, P = 0.004). **f.** Gazing-like ratio (*n* = 7, 6, 7; P = 0.271). **g.** Latency to object recognition (n = 7, 6, 7; P = 0.659). **h.** Object touching durations for early (0–2.5 min) and late (2.5–5 min) phases of the object + session. Dark blue, interactively moving object; light blue, regularly moving object; gray, static object (*n* = 7 for static, 6 for regularly moving, 7 for interactively moving; early phase, P = 0.172; late phase, R vs. S, P = 0.067; IT vs. R, P = 0.032; IT vs. S, P < 0.001). Circles and error bars represent mean ± SEM. **d-g.** Black circles in the bar graphs indicate individual data. Error bars represent SEM. n.s. indicates not significant.

observations made using real objects that repetitive exposure to an object reduces the motivation to interact [24]. Collectively, our results indicate that the interactive movement of the visual object made the rats maintain their interest for a prolonged time.

Next, we determined whether or not the preference for an interactively moving object was due to a simple visual flow. To test this, we presented an interactively moving background pattern that, when touched, was dislocated at the same velocity, distance and direction exhibited by the interactively moving object (Fig. 3a). Our results showed that the rats stayed about 2-fold longer in the interaction zone when presented with the interactively moving target versus the interactively moving background (Fig. 3b, c; background, 62.292 ± 4.818 s; target, 115.989 ± 13.350 s; P = 0.001). The object-touching duration was 2-fold longer for the interactively moving target compared to the interactively moving background (Fig. 3d; background, 16.691 ± 2.867 s; target, 34.067 ± 5.929 s; P = 0.014), whereas there was no significant difference in the level of visual salience (Fig. 3e; background, 0.410 ± 0.073; target, 0.322 ± 0.040; P = 0.310). These results suggest that the rats exhibited differential recognition of the target object versus the background and paid more attention to the movement of a target than to the overall optical flow.

The time in interaction zone of static was slightly longer for background moving (Figure 3c, 62.292 ± 4.818 s; *n* = 8) than static (Figure 2d, 43.756 ± 7.527 s; *n* = 7), even there was no significant difference between these two (two-tailed unpaired *t*-test, t = 2.129 with 13 degrees of freedom, P = 0.0530). Also, time in interaction zone was slightly longer in interactively moving target with background (Figure 3c, 115.989 ± 13.350 s; *n* = 6) than without background (Figure 2d, 90.508 ± 9.808 s; *n* = 7), even there was no significant difference (two-tailed unpaired *t*-test, t = 1.568 with 11 degrees of freedom, P = 0.145). These were consistent to object touching durations. The object touching duration was slightly longer in background moving (Figure 3d, 16.691 ± 2.867 s; n = 8) than static (Figure 2e, 14.793 ± 2.958 s; n = 7), but there was no significant difference (two-tailed unpaired *t*-test, t = -0.460 with 13 degrees of freedom, P = 0.653). The object touching duration of interactively moving target with patterned background (34.067 ± 5.929; *n* = 6) was slightly longer than interactively moving target without background (32.445 ± 3.129 s; *n* = 7), still there was no significant difference (two-tailed unpaired *t*-test, t = -0.252 with 11 degrees of freedom, P = 0.805). These results indicate that the difference between background and target was not due to the increase of interaction time with the target. We could also conclude that the patterned background was not enough to make a significant difference on the interest of the rats to the object.

Given that rodents show defensive behaviour in response to an enlarging visual stimulus that may be perceived as an approaching predator [3], we hypothesized that rats would preferentially approach an interactively shrinking object, which would mimic a prey fleeing in response to a perceived predator (Fig. 4). Indeed, an interactively enlarging object (Fig. 4a) elicited instant vigilance behaviour in all rats (Fig. 4b, c, *see Video S5*), whereas an interactively shrinking object yielded more attracted responses (Fig. 4d-f, *see Video S6*). The locomotor velocities of rats interacting with the enlarging object were significantly faster than those of rats interacting with shrinking object because of the vigilance behaviours (Fig. 4g; static, 8.661 ± 0.973 cm/s; enlarging, 11.403 ± 1.578 cm/s; shrinking, 4.474 ± 0.888 cm/s; static vs. enlarging, P = 0.113; enlarging vs. shrinking, P = 0.003; static vs. shrinking, P = 0.041). The rats interacted significantly longer with the interactively shrinking object in terms of time spent in the interaction zone (Fig. 4h; static, 43.756 ± 7.527 s; enlarging, 50.565 ± 5.962 s; shrinking, 117.694 ± 21.7 s; static vs. enlarging, P = 0.745; enlarging vs. shrinking, P = 0.009; static vs. shrinking, P = 0.005) and object touching (Fig. 4i; static, 14.793 ± 2.958 s; enlarging, 17.061 ± 2.301 s; shrinking, 36.967 ± 4.320 s; static vs. enlarging, P = 0.627;

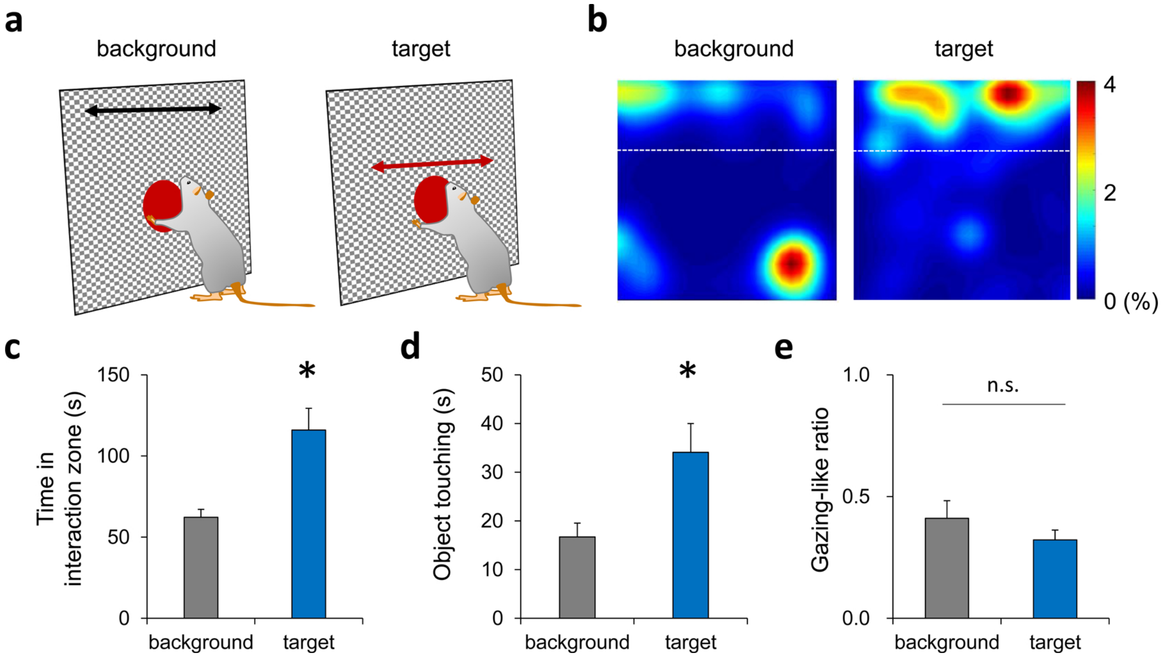

**Fig. 3. The presence of an interactive object, but not an interactive background, induces active engagement. a.** Schematic figure of the background-moving and target-moving sessions. **b.** Representative heatmap of the time spent. The dashed line indicates the boundary of the interaction zone. **c.** Time spent in the interaction zone during the moving-background and moving-target sessions ($n$ = 8, 6; P = 0.001). **d.** Object-touching duration for moving-background and moving-target sessions ($n$ = 8, 6; P = 0.014). **e.** Gazing-like ratio for moving-background and moving-target sessions ($n$ = 8, 6; P = 0.310). Error bars represent SEM. Black circles in the bar graphs indicate individual data. n.s. indicates not significant.

enlarging vs. shrinking, P = 0.001; static vs. shrinking, P < 0.001), whereas there was no significant difference in the gazing-like ratio (Fig. 4j; static, 0.370 ± 0.070; enlarging, 0.351 ± 0.059; shrinking, 0.372 ± 0.053; P = 0.969). Taken together with our earlier finding that rats were more interested in the interactively moving object, these results suggest that the rats highly react to the object when it exhibits escaping-like movement.

## 4. Discussion

Here, we report that the rats show more attraction to visual features that moves interactively with respect to their behaviour, compared to static or regularly moving objects, and that they show less preference for, and sometime escape behaviour relative to, an object that enlarges in an interactive manner. To the best of our knowledge, this is the first report of precise and reproducible visual features that elicit approaching and exploratory behaviour in rats. Our findings have a number of implications.

First, the phenomenon we found seems to be correlated to the play behaviour of animals, consisted of hunting-like actions that are structurally indistinguishable from hunting behaviour in terms of their motions and sequences [8]. Indeed, play behaviour has been considered to represent hunting behaviour toward inedible targets because the animals have been found to prefer playing with prey-like objects that resemble their targets in size and texture [8,25]. In this sense, we provide a conceptual connection between hunting behaviour and object interaction behaviour. In terms of the predator-prey arms race, predators need to develop a tactic for spotting their prey as quickly and efficiently as possible. Even before they confirm that a visually reactive object is a prey, they need to focus and chase the object. Indeed, recent studies on hunting behaviour found that the optogenetic stimulation of hunting circuits induces hunting-like behaviours even towards non-prey objects [14–16]. This means that regardless of whether the object is prey or non-prey, the predator must trigger and orchestrate hunting actions based on visual features of the object. Overall, our results support the idea that object playing in animals might represent the same action as hunting and that, evolutionarily, playing might enable the animals to practice their hunting skills and motions [8].

Second, our results suggest that the prey's visible reaction to predatory exploration causes the predator to focus on and chase the prey. In this context, it makes perfect sense that the prey animals show freezing behaviour [26] and may even play dead [27] in order to minimize their visual salience. Using this sort of adaptive behaviour, prey animals can reduce their chances of being detected by nearby predators. However, when a prey animal recognizes that a predator correctly spot it, the prey animal should flee as quickly as possible. Consistent with this, studies have shown that mice instantly run from fast-approaching visual stimuli that mimic aggressively charging avian predators, whereas they freeze in the presence of slowly passing visual stimuli that mimic predators located far away in the sky [26]. Together, our results and the previous findings imply the idea that prey animals develop stealth-based adaptive behaviours in response to the predator's ability to discriminate reactively moving prey.

Third, our results indicate that the virtual object system described herein provides a robust paradigm for studying the neural mechanism of predatory or object interaction behaviours in terms of visual perception. Even though the vision could be the most important sensory signal for predatory exploration in rodents [18], the vision-specific functions cannot be effectively studied using real prey because of its uncontrollability. Further studies using our testing paradigm could focus on revealing the anatomical and functional mechanisms of visual hunting in animals because our system uses controllable visual signals. A recent study revealed that the medial preoptic area (MPA) sends a signal via the axonal projection to the periaqueductal gray (PAG) to elicit the motivation for object-directed behavior and active hunting behavior [28]. Interestingly, stimulation of the MPA-PAG triggered mice to persistently interact with a reactive 3D object, but their interest in a non-reactive 2D object quickly waned under the same stimulation [28]. This result and our present findings suggest that object reactivity is needed to maintain the animal's exploration of an object. However, we do not currently know how the sensory input of the target object or prey triggers and maintains the motivation for these behaviors. It seems

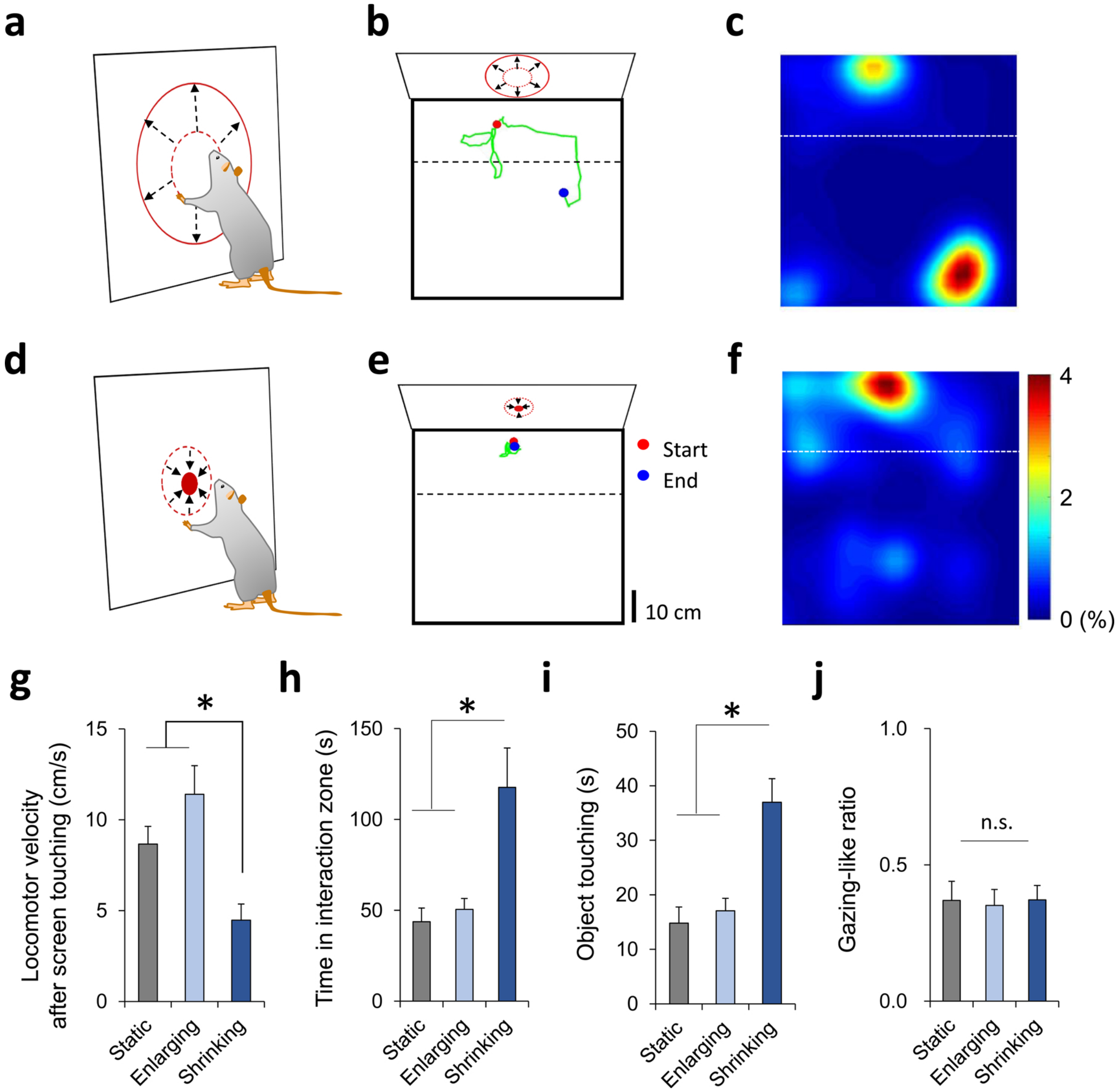

**Fig. 4. Rats exhibit hyper-responsiveness to an interactively shrinking target. a.** Schematic figure of the interactively enlarging object. **b.** Representative track for 5 s after it touched the interactively enlarging object. **c.** Representative heatmap of time spent during the presentation of the interactively enlarging object. **d.** Schematic figure of the interactively shrinking object. **e.** Representative track for 5 s after it touched the interactively shrinking object. **f.** Representative heatmap of time spent during presentation of the interactively shrinking object. **g.** Locomotor velocity of rats for 5 s after screen touching (n = 7, 6, 6; static vs. enlarging, P = 0.113; enlarging vs. shrinking, P = 0.003; static vs. shrinking, P = 0.041). **h.** Time spent in the interaction zone (*n* = 7, 6, 6; static vs. enlarging, P = 0.745; enlarging vs. shrinking, P = 0.009; static vs. shrinking, P = 0.005). **i.** Object-touching duration (*n* = 7, 6, 6; static vs. enlarging, P = 0.627; enlarging vs. shrinking, P = 0.001; static vs. shrinking, P < 0.001). **j.** Gazing-like ratio. Dotted line represents the mean value obtained from the static object experiment (*n* = 7, 6, 6; P = 0.969). Error bars represent SEM. Black circles in the bar graphs indicate individual data. n.s. indicates not significant.

likely that a feedback neural loop that acts via the retina recognizes the visual reaction to the target object and feeds neural activity to components of the motivation circuit, such as the MPA-PAG, in order to prolong the animal's desire to interact with and explore an object. A strong candidate for this visual feedback circuit would be the superior colliculus (SC), for three reasons: first, the SC neurons receive direct retinal projections in a topographical manner [29] and send their projections to the PAG [30]; second, the SC can recognize visible movement, such as that of a prey [31]; and third, the SC region has been shown to be crucial for successful hunting [30]. It is possible that the visible reaction of an object activates the SC neurons, which activate the PAG neurons to maintain the motivation for object exploration or hunting. Finely tuned motor outputs for the behaviors might be regulated elsewhere. At the very least, our system offers a convenient behavioral assay that researchers may use to uncover the underlying neural mechanisms through which a visual cue affects the motivation for object exploration and hunting in rodents.

Also, future studies using our methodology may illuminate additional visual features, such as color, shape, size and/or brightness that affect an animal's interest in foraging and hunting. This behavioural paradigm will open new opportunities for studying the neural circuits that underlie the exploration aspects of hunting and foraging behaviour.

## CRediT authorship contribution statement

**Daekyum Kim:** Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation, Writing - original draft. **Yong-Cheol Jeong:** Conceptualization, Data curation, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Chanmin Park:** Data curation, Formal analysis, Investigation. **Anna Shin:** Data curation, Formal analysis, Investigation. **Kyoung Wan Min:**

Data curation, Formal analysis, Investigation. **Sungho Jo:** Funding acquisition, Project administration. **Daesoo Kim:** Conceptualization, Funding acquisition, Project administration, Writing - review & editing.

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