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Automated scoring of novel object recognition in rats

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ABSTRACT

The object recognition task (ORT) has become increasingly popular as a memory test in neuroscience research. Scoring of ORT performance is still mostly done by hand, which can be liable to subjective scoring. To our knowledge, no suited software is available yet since the direction of the nose of the animal cannot be tracked reliably. We have developed a software paradigm that reliably tracks the nose of the rats and have conducted a series of experiments to evaluate the reliability of this newly developed program. We used Wistar rats, which showed good object memory after 1 h interval. Subsequently, we used scopolamine (SCOP) to impair the memory performance of the rats. The object exploration was scored by two observers and the automated system. Both observers and the automated system found an impairing drug effect of scopolamine on ORT performance. When using large objects the correlation between the discrimination index d2 of observers was: 0.60 (SCOP) and 0.79 (SAL). However, the correlation between observers and the automated system was quite low: 0.41 (SCOP) and 0.40 (SAL). Reducing the size of the objects increased the reliability between observers and the automated system substantially (0.82–0.87). We conclude that the use of small objects in combination with our program enables reliable automated scoring in the ORT, thus increasing the objectivity and validity of this task.

1. Introduction

The object recognition test (ORT) is a behavioral test that is widely used to examine animal memory performance. This test was first described by Ennaceur and Delacour (1988) and has been used in many different variations ever since (e.g. Mumby, 2001; Prickaerts et al., 1997). A large amount of studies have used the ORT for behavioral assessment of learning and memory, e.g. a search using PubMed shows over 1550 behavioral studies using variations of the ORT.

The ORT has been shown to be an effective model to assess learning and memory across species provided suitable strains are selected (Şık et al., 2003), i.e. the literature reports behavioral studies of ORT performance in mice (e.g. Şık et al., 2003), hamsters (e.g. Palchykova et al., 2006), rats (e.g. Blokland et al., 1998) and pigs (e.g. Moustgaard et al., 2002).

Memory performance in the ORT is based on the natural tendency of animals to explore novel objects. An important advantage of this task is that no aversive/stressful stimuli need to be applied. A further advantage of the ORT is that it is a two-trial paradigm. This opens the possibility to assess the effects of putative cognition-modulating compounds on the acquisition, the consolidation, and the retrieval of memory (e.g. de Lima et al., 2005; Lamirault and Simon, 2001; Prickaerts et al., 2005), and to further analyze the precise dynamics of the consolidation process (e.g. Rutten et al., 2006). Furthermore, each animal can be repeatedly tested under the same, or modified experimental conditions (e.g. different retention intervals, different doses of a test compound) and such a within subject design allows a reduction of the required number of animals needed in an experiment.

A major drawback of the ORT is that the scoring is done by manual scoring, which can be liable to subjectivity. Although the observer is usually blind for the treatment conditions, the expectancy of the observer might still influence behavioral scoring. Furthermore, in certain studies (for example in studies with knockout animals that have a distinct physical appearance) it is practically impossible to be completely blinded for the treatment conditions or experimental setup.

Automation of the ORT would greatly reduce the time to complete studies since two or more animals could be tested simultaneously in parallel automated setups. Furthermore and more importantly, the issue of subjectivity is completely abolished when

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an automatic system scores animal behavior. Various attempts have been made to automate the scoring of the rats, but to our knowledge no reliable software is available or published yet. One problem that may hamper the development of such an image analysis system is related to the ability to reliably track the nose of the animal. Although, commercially available software packages can detect and track the center mass point of animals in an arena, this is not appropriate for scoring the ORT. In order to successfully score exploration behavior in the ORT the software must detect and track the nose of an animal.

Recently, we developed an automatic scoring paradigm that reliably tracks the nose of the rats. The aim of the present study was to compare and validate this scoring paradigm by comparing the automatically obtained scores with the manually obtained scores of two experienced independent observers. Of note, we compared the automatic and manual scoring correlations of a set of small objects with a set of large objects for validation purposes of the automated system. Also, we evaluated drug effects in order to evaluate the sensitivity of the program to detect changes in memory performance. We expected that the exploration times and discrimination measures (d2) obtained through our automatic scoring paradigm will not differ from manual scoring by trained observers.

2. Materials and methods

2.1. Animals

All experimental procedures were approved by the local ethical committee of the Maastricht University for animal experiments according to governmental guidelines. A total of 24 young adult male Wistar rats (Harlan, The Netherlands) were used in this study. The animals were housed individually in standard type 3 Makrolon cages on sawdust bedding in an air-conditioned room (about 20 °C). They were kept under a reversed 12/12-h light/dark cycle (lights on from 18.00 to 6.00 h) and had free access to food and water. Rats were housed in the room where they were tested. A radio that played softly provided background noise in all rooms. All testing was done between 9.00 and 17.00 h.

2.2. Object recognition memory

The novel object recognition test was performed as described previously (Prickaerts et al., 1997). The apparatus consisted of a circular arena, 83 cm in diameter. Half of the 40 cm high wall was made of grey (RAL 7035) polyvinyl chloride, the other half of transparent polyvinyl chloride. The test room was dimly lit by a small lamp (60 W), located in a corner of the room. Furthermore, the arena was illuminated with a spotlight (60 W) dimmed with a white paper filter, this generated a light intensity of approximately 8 lx that was equal in all parts of the floor of the apparatus. The floor of the arena was also made of grey (RAL 7035) polyvinyl chloride which allows detection of both black and white animals with a video-tracking system. Furthermore, the surface of the floor was abraded to reduce interference by reflections. Two objects were placed in a symmetrical position about 10 cm away from the grey wall. We used two groups of four different sets of objects, one group consisted of large objects and the other group consisted of smaller objects. In the large object group the different sets of objects were: (1) a cone consisting of a grey polyvinyl chloride base (maximal diameter 18 cm) with a collar on top made of brass (total height 16 cm), (2) a standard 1-l transparent glass bottle (diameter 10 cm, height 22 cm) filled with sand, (3) a massive metal cube (10.0 cm × 5.0 cm × 7.5 cm) with two holes (diameter 1.9 cm), and (4) a massive aluminium cube with a tapering top (13.0 cm × 8.0 cm × 8.0 cm). In the small objects group the different sets of objects were: (1) a square glass bottle with rounded corners filled with concrete (5 cm × 5 cm × 6.5 cm), (2) a plastic standard laboratory 50 ml tube (Greiner) filled with concrete (diameter 3 cm, height 11.5 cm), (3) a massive metal cube (2.5 cm × 5 cm × 7.5 cm) with two holes (diameter 1.5 cm), and (4) a massive aluminium cube with a tapering top (4.5 cm × 4.5 cm × 8.5 cm). The objects could not be displaced by a rat.

In the first week, the animals were handled daily and were adapted to the observation arena on 2 successive days, i.e. they were allowed to explore the arena (without any objects) twice for 3 min each day. In the next 2 weeks the rats were adapted to the testing and intra-peritoneal (i.p.) administration procedures by a saline injection (0.4 ml) 30 min before the first trial until they showed a stable discrimination performance, i.e. good object discrimination at a 1-h interval.

A testing session comprised two trials and the duration of each trial was 3 min. During the first trial the apparatus contained two identical objects (samples). A rat was always placed in the apparatus facing the wall in the center of the transparent front segment. After the first exploration period the rat was put back in its home cage. Subsequently, after a delay interval, the rat was put back in the apparatus for the second trial, but now with two dissimilar objects, a familiar one (the sample) and a new one. The times spent exploring each object during the first and the second trial were recorded manually by two experienced independent observers using two personal computers.

Exploration of an object was defined as follows: directing the nose to the object at a distance of no more than 2 cm and/or touching the object with the nose. Sitting on the object was not considered exploratory behavior. The times spent exploring each object during T1 and T2 were recorded manually using a personal computer. This was done by two independent observers.

In order to avoid the presence of olfactory trails the objects were thoroughly cleaned after each trial. Moreover, each object was available in triplicate so none of the two objects from the first trial had to be used as the familiar object in the second trial. In addition, all combinations and locations of objects were used in a balanced manner to reduce potential biases due to preferences for particular locations or objects.

Rats were trained and tested using a 1-h delay interval between the first and second trial. Normally Wistar rats show good discrimination between the two objects after this interval (Rutten et al., 2006). Each week three testing sessions were performed (Monday/Wednesday/Friday), and a washout period of at least 48 h between tests was taken into account.

2.3. Object recognition software

To score the exploration behavior of the rats automatically, a camera (Sony CCD-IRIS, B/W camera, PAL) was mounted above the center of the arena. The camera was connected with a PC (Windows XP platform, 2.6 GHz) using a frame grabber card (IMAQ PCI-1411; National Instruments). The user-interface and processing algorithms were developed using LabVIEW 7.1 with additional specific modules added (VI Technologies, Weert, The Netherlands). The image on the PC monitor could be adjusted by manipulating the intensity threshold to obtain a clear image of the arena and the objects therein. It is essential that the lighting of the arena and the proximity of the objects are equal to prevent shades that may interfere with reliable tracking of the animal. Regions of Interest (ROI) could be selected to indicate the position of the objects. In our experiments feces or urine did not interfere with the detection of the animal at all. The detection paradigm ignores these small reflections. The system searches for larger rat-shaped formations.

The processing and detection of the nose of the animal is shown in Fig. 1. The detection algorithm of the nose was as follows. First,

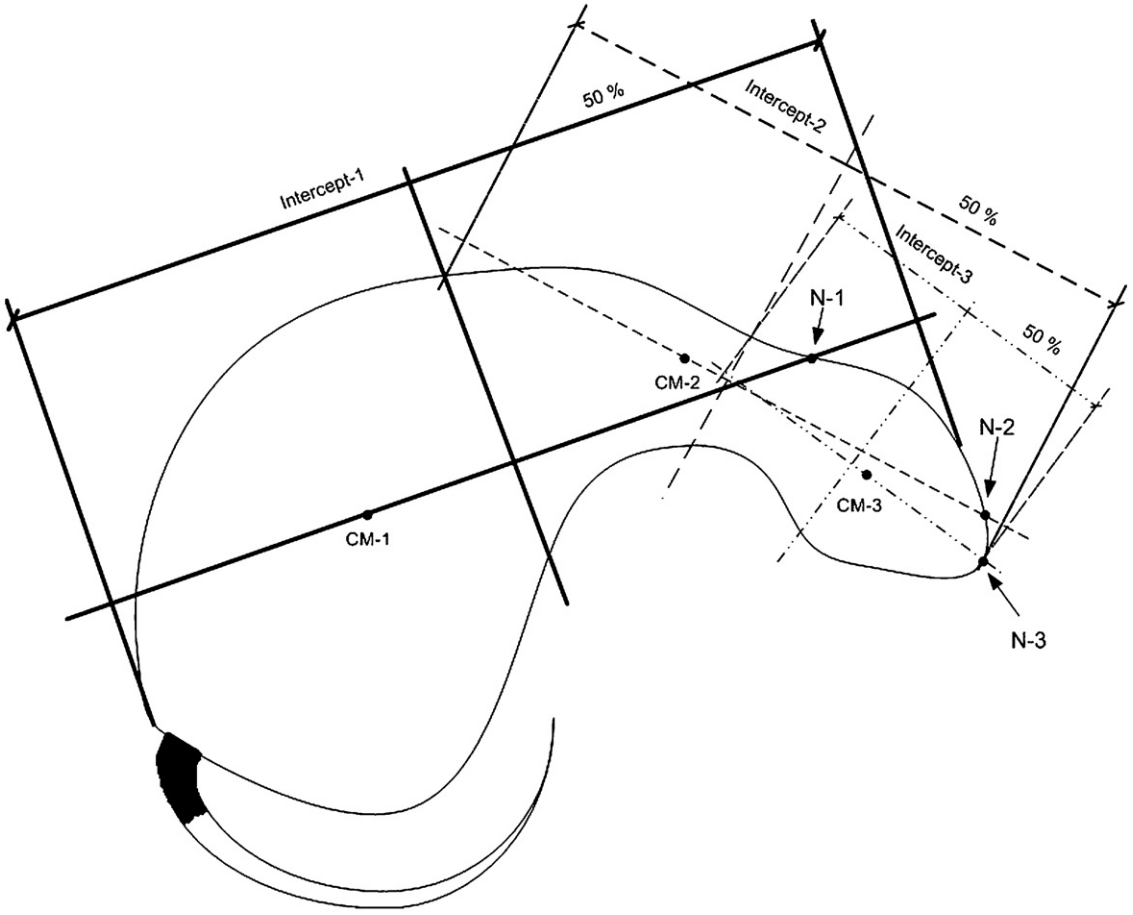

Fig. 1. Detection algorithm of the nose of a rat. See text for details of algorithm.

two objects were determined, a small (tail) and a large (body) one. This was enabled by marking the tail of the rat. Next, the maximal intercept of the rat was determined. Subsequently the Center of Mass (CM-1) was determined and the line of the maximal intercept was shifted so that the maximal intercept line crossed the Center of Mass (see solid lines in Fig. 1). This resulted in three different *X*–*Y* coordinates: Center of Mass, intercept-body crossing front, intercept-body crossing back. Then, a perpendicular was placed on 50% of the intercept line. The perpendicular divided the body in two parts and the Center of Mass was again calculated for the front part (CM-2). The front and the back of the animal were determined by the position of the tail. Thus, the nose position was determined as being on the opposite side of the small (i.e. tail) object. The point where the maximal intercept crossed the perimeter of the body was regarded as the position of the nose (N-1, see Fig. 1). Using this algorithm, which was executed in a cycle of 40 ms, the nose gradually shifts to the actual nose position (see shifts from N-1 (solid lines) to N-3 (dotted-dashed lines) in Fig. 1). This process is dynamic since the *X*–*Y* coordinates of the previous calculation are compared with the new input of *X*–*Y* coordinates. Thereby, this process continuously leads to the detection of the actual nose position.

If the nose position fell within the ROI, a timer was started to measure the time the nose was in the ROI. The times the nose was in the ROI were cumulated and resulted in a time spent in the two different ROIs for each object separately. For people that are interested in acquiring a copy of the software implementation described above, further information can be obtained from the authors via email. A screen shot of the user-interface obtained during real-time tracking of a rat in the object recognition task is provided in Fig. 2.

2.4. Treatment

Scopolamine (SCOP) HBr (0.1 mg/kg) was dissolved in saline (0.9% NaCl in water). Saline or scopolamine was administered i.p., in a volume of 1 ml/kg, 30 min prior to the first trial in the ORT.

2.5. Statistical analysis

The basic measures were the times spent by rats in exploring an object during trials 1 and 2. Table 1 shows the measures involved in the object recognition task (Prickaerts et al., 1997). e1 and e2 are measures of the total exploration time of both objects during trials 1 and 2, respectively. d2 is considered as an index measure of discrimination between the new and the familiar objects. In fact, d2 is a relative measure of discrimination which corrects the difference between exploring the old and the novel object for total exploration activity (e2). Animals that did not explore sufficiently in the second trial (i.e. less than 5 s) were excluded from the data analysis for that

Table 1
Inter-observer correlations for the measure e1, e2 and d2

Conditions	Objects	r^2		
		e1	e2	d2
Saline	Small	0.959	0.953	0.727
	Large	0.909	0.758	0.778
Scopolamine	Small	0.841	0.939	0.854
	Large	0.774	0.802	0.610

e1 = Exploration time in trial 1; e2 = exploration time in trial 2; d2 = discrimination index.

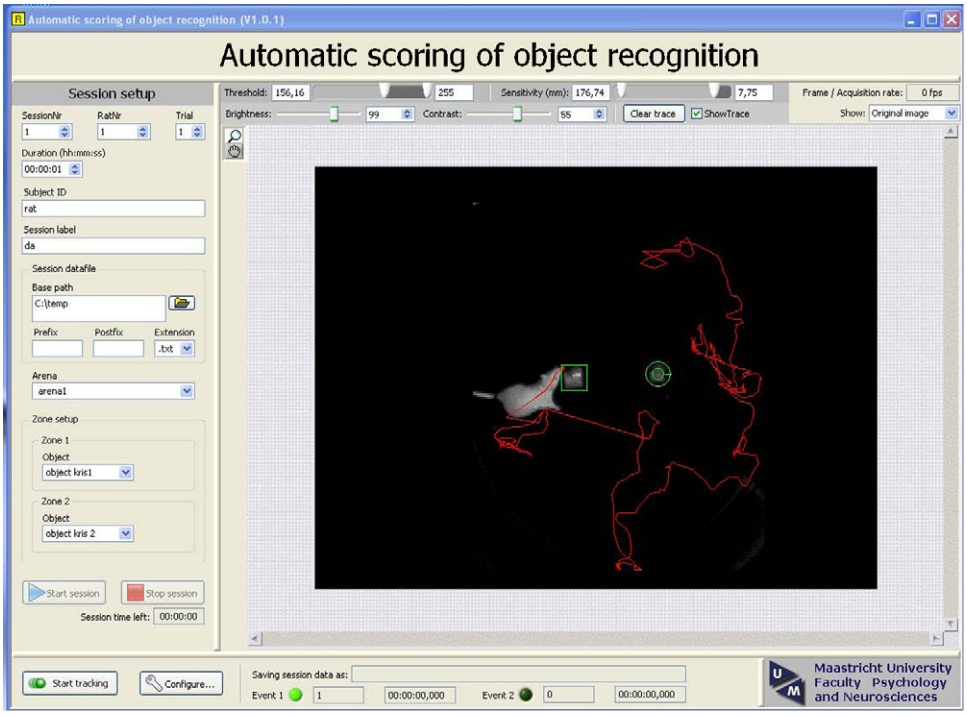

Fig. 2. A screen shot of the user-interface obtained during real-time tracking of a rat in the object recognition task. In the left panel, all experimental parameters (e.g. session length, rat nr, trial nr, etc.) can be entered. Furthermore, the different arenas and the object-ROIs can be selected from a list or customized manually. The large panel on the right shows the live video feed of the rat in the arena, two objects with their corresponding ROIs and a trace of the distance traveled by the animal. In the panel above the video feed the detection threshold, brightness and contrast can be adjusted. Furthermore the sensitivity, i.e. minimum and maximum distance moved (mm) between two frames, can be customized. The bottom panel displays the number and total duration of exploration of the left (event1) or right (event2) object.

particular test session. Treatment effects on d2 values were examined with Student's *t*-tests ($P < 0.05$). Pearson correlations (r) were calculated between the two observers and the automated system.

3. Results

In general, the correlations between the two independent observers in all conditions were highly significant (see Table 1) Therefore, the averaged measures of the ORT (e1, e2 and d2) of the two observers were used for comparison with the automatic scores in the next section.

The averaged exploration times in the first (e1) and second trial (e2) of the two observers versus the automatic scores are depicted in Fig. 3. The correlations between manual scoring and automatic scoring when using small objects in the ORT were high and significant ($r \geq 0.60$, see Fig. 3, left panel). However, when large objects

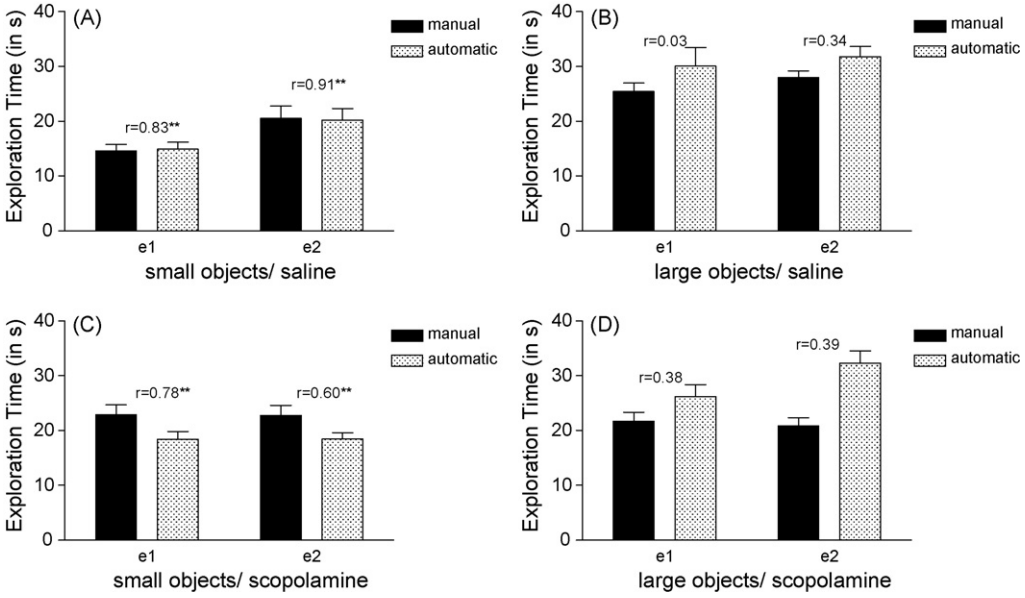

Fig. 3. Manually or automatically acquired exploration times for trail 1 (e1) and trial 2 (e2) in the object recognition task. Animals were tested using small or large objects and treated with either saline or scopolamine 30 min before trial 1. (A) small objects and saline, (B) large objects and saline, (C) small objects and scopolamine, and (D) large objects and scopolamine. Pearson correlations between manual and automatic scores are depicted above each trail.

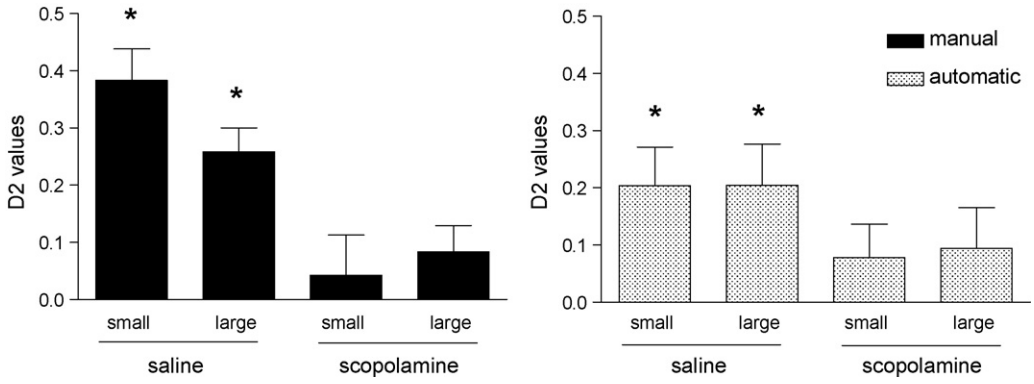

Fig. 4. Discrimination performance (d2) after treatment with saline or scopolamine (30 min before T1) in sessions with small or large objects. Manually obtained d2 values (left panel) and automatically obtained d2 values (right panel) are compared to zero. Asterisks indicate significant differences from zero (*$P < 0.05$).

were used the correlations were small and non-significant ($r \leq 0.39$, see Fig. 3, right panel).

When comparing the correlations for the d2 values we found when using large objects the correlation between the observers was high: $r = 0.79$ in the saline treatment condition and $r = 0.60$ in the scopolamine treatment condition. However, the correlation between observers and the automated system was quite low: $r = 0.40$ for the animals treated with saline and $r = 0.41$ for the animals treated with scopolamine. When small objects were used the correlation between observers was also high: $r = 0.87$ in the saline condition and $r = 0.83$ in the scopolamine condition. Furthermore, the correlation between observers and the automated system was improved significantly: $r = 0.82$ in the saline condition and $r = 0.85$ in the scopolamine condition (data not shown).

Fig. 4 shows the effects of saline versus scopolamine treatment on the discrimination performance d2 when tested with either small or large objects. The left panel of Fig. 4 shows the average d2 values of the two observers after manual scoring. The d2 values of saline treated animals were significantly higher than zero for both small ($t(19) = 7.03$; $P < 0.01$) and large objects ($t(17) = 6.18$; $P < 0.01$). Scopolamine treated animals showed memory impairment when tested with either small or large objects, i.e. d2 values did not differ from zero for small objects ($t(21) = 0.60$; n.s.) or large objects ($t(21) = 1.80$; n.s.).

The d2 values based on the automatic scores are depicted in the right panel of Fig. 4. A similar picture as the manual scoring was observed, although d2 values in general were lower in the automatically scored versus the manually scored data. The d2 values of saline treated animals were significantly higher than zero for both small objects ($t(19) = 3.00$; $P < 0.01$) and large objects ($t(17) = 2.87$; $P < 0.05$). Finally, scopolamine treated animals showed memory impairment when tested both with small objects ($t(21) = 1.36$; n.s.) or large objects ($t(21) = 1.34$; n.s.), i.e. d2 values did not differ from zero (see Fig. 4 right panel).

4. Discussion

In the present study we examined the reliability of an automated scoring system to measure object exploration and recognition in rats. Rats were trained in the ORT and tested after treatment with saline or scopolamine. We used two sets of objects, small objects and large objects. In the sessions with large objects, relatively poor correlations between the exploration times of the manual scores and the automatic scores were found. However, when using small objects the correlations between manual and automatic scoring increased substantially. Furthermore, both observers and the automated system were able to measure an impairing drug effect of scopolamine on ORT performance.

The data presented above show that using an automated system for the assessment of exploratory behavior in the ORT highly correlates with the manually obtained scores by two independent observers. The correlation between automated and manually scored exploration times was highly influenced by the size of the objects used. This could be explained by the definition of exploration behavior that was used to score exploration in the ORT. For manual scoring, exploration behavior was defined as follows: directing the nose to the object at a distance of no more than 2 cm and/or touching the object with the nose. Sitting on the object was not considered exploratory behavior. Thus, if an animal stood against or leaned on an object and had its nose more than 2 cm above the object this would not be scored by the manual observers.

However, the automated system records from above and cannot discriminate in a three-dimensional manner. Therefore the automated system would score this type of behavior as exploration. Using large objects increases the likelihood that animals will stand up against the objects or lean over them, which evokes a dramatic increase in false positive exploration scores. The small set of objects reduced rearing and leaning behavior and therefore, the automated system scored the actual exploration behavior more accurately and reliably.

Scopolamine is known to impair memory performance in various behavioral tasks (e.g. Blokland et al., 1992; Devan et al., 2004; Imanishi et al., 1997; Zhang and O'Donnell, 2000) and the present study corroborates the memory impairing effects of scopolamine in a 1-h delay in the ORT (Lieben et al., 2005; Rutten et al., 2006, 2007). Furthermore, we demonstrated that the novel-automated scoring system for the ORT can equally detect pharmacological manipulations in memory performance.

Although behavioral testing can be performed in a double blind setting, the observer might be biased for a certain location or object. Automation of the scoring procedure rules out subjectivity in the behavioral assessment of cognition enhancers or cognition impairers in the ORT. Furthermore, automation of scoring allows simultaneous behavioral assessment in multiple arenas. This may significantly increase the speed and efficiency of compound testing.

When correlations of the d2 values between observers were compared, it did not matter whether small or large objects were used (>0.60). However, when comparing the d2 index of the observers with the automated system the size of the objects was of great importance. Using large objects correlations between observers and the automated system were quite low (<0.41). Reducing the size of the objects increased the correlations substantially (>0.82). Although individual differences between observers will always exist, high correlations between manual and automatic scoring of the measures of the ORT indicate that the automated system can assess discrimination performance to an equal extent

as manual observers would. Nevertheless, the main advantage of the automated system is that exploration scores are completely objective without subjective observer-based biases.

To our best knowledge, no reports of nose-tracking object recognition software programs have been reported in the current peer-to-peer reviewed literature. Of note, recently a short communication was published in Journal of Neuroscience Methods, claiming a method for automation of the novel object recognition (Silvers et al., 2007). However this method did not detect the nose of an animal and relied on photo beam crossings in an open field.

In conclusion, our results show that the automated scoring system reliably assesses exploratory behavior in the ORT. Note that small objects are preferred in order to avoid overestimation of exploratory behavior which may lead to false positives and invalid conclusions from the behavioral data. This automated system can objectively score memory performance in the ORT in rats. This system could also be applied to alternative versions of the ORT, such as the object location test (e.g. Murai et al., 2007; Trippodi and Rose, 2003). Furthermore, the automated system is currently being validated in the ORT for mice. Employment of the automated system would enhance the reliability and objectivity, as well as efficiency and speed of memory assessment in the ORT.

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