Assessing working memory for objects in rats: no one said it was easy

(A letter pertaining to the paper by Herremans, Hijzen and Slangen on pages 1963-1965 of this issue)

Dear Sir,

Herremans et al conclude that the DNMS task, as described by Mumbey, Pinel and Wood¹ cannot be used to assess working-memory in rats. However, their experiment does not demonstrate that at all. What it does demonstrate is the difficulty of properly administering non-automated memory tasks to rats and avoiding confounds that enable them to circumvent the intended cognitive demands.

Soon after we described our DNMS paradigm,¹ we realised that our procedures had the potential to introduce the same confound that Herremans et al claimed to have demonstrated. Like Herremans et al, we were concerned with the possible confounding effects of odours left on the stimulus objects by the experimenter, who must handle them on each trial. We conducted a series of experiments to determine whether such experimenter-odour cues might be influencing our results.² In contrast to the conclusions of Herremans et al, our experiments indicated that rats do not use touch-produced experimenter-odour cues to solve the DNMS task, at least not in our hands.

The hypothesis is that rats solve the DNMS task by an olfactory discrimination based on the relative recency with which the sample and novel objects are touched by the experimenter. Our original DNMS procedure might have permitted such a solution because the sample object was always handled more recently than the novel object prior to the choice phase of each trial.¹ One prediction that follows is that rats will be unable to solve the DNMS task if the sample and novel object are each touched last on a random half of the trials. At the very least, acquisition should be significantly slower under these conditions than with the procedure described by Mumbey et al,¹ in which the sample is always touched last. We compared the DNMS acquisition rates of rats trained under these two conditions.² Our rats clearly did not benefit from the sample being handled more recently than the novel object. Rats mastered the DNMS task in both conditions, and there was no significant difference in the number of trials required to reach an 85%-correct performance criterion under the two conditions. In fact, the difference between the two groups was in a direction opposite to that predicted. The rats for which the sample was always placed in the choice area last required an average of 460 (SE = 44.0) trials to achieve the criterion, whereas the rats in the random-placement group required an average of 405 (SE = 9.6) trials.² Importantly, these acquisition rates were similar to those in previous experiments.¹,²,³,⁴

The foregoing results demonstrated that rats can solve the DNMS task without discriminating touch-produced odour cues, but the possibility remained that rats trained in the procedure that enabled such a discrimination had in fact been solving the task this way. Probe tests indicated that they were not. After the rats reached criterion with the procedure in which the sample object was always handled last, we changed the procedure so that the order of placement of the sample and novel objects varied randomly across trials. There was no effect on DNMS accuracy. Thus, even rats that received all of their initial DNMS training with the sample always being handled last did not behave as though they could discriminate between the objects on that basis.

Clearly, our rats were not solving the DNMS task by discriminating which object was touched most recently by the experimenter. However, this does not preclude the possibility that this potential confound will prevail under circumstances and in some laboratories. Accordingly, we conducted another experiment to determine whether rats could in fact learn to make such a discrimination if they were expressly trained to do so. A set of 40 plastic bottle lids, identical in appearance, served as the stimulus objects. Each one was placed over one of the food wells behind the closed door, this lid, by virtue of having been touched first by the experimenter, was designated S⁺ (rewarded). Approximately 4 sec following placement of S⁺, the experimenter placed a second lid over the vacant food well, beside S⁺; this second lid was designated S⁻ (not rewarded). The only consistent difference between S⁺ and S⁻ was that the S⁺ always handled about 4 sec before S⁻. The 4 sec intertouch interval is roughly equivalent to the interval between when the sample and novel objects are touched by the experimenter with the DNMS procedures described by Mumbey et al.¹ Different pairs of lids were used on successive trials, and all 40 lids were washed after each session in order to eliminate any extraneous scents that they might have acquired.

Remarkably, each rat eventually solved the discrimination task. A special probe procedure, and observation of rats’ behaviour while the experimenter positioned the objects, indicated that they were not using auditory or visual cues associated with the order of placement. It was concluded that the rats were indeed discriminating the objects on the basis of touch-produced experimenter-odour cues. However, the discrimination task was much more difficult to learn than the DNMS task, and when results from the two tasks were compared it was again clear that a strategy based on this discrimination had not been supporting our rats’ DNMS performance. For instance, rats required significantly more trials to master the discrimination task (M = 605.0) than they required to master the DNMS task to the same 85% criterion (M = 432.5). Furthermore, 75% of the rats solved the discrimination only after they received 100 correction trials, which were administered because these rats were still performing at chance levels after 400 trials. Most rats attain high accuracy levels on the DNMS task in fewer than 400 trials, and without the benefit of additional correction trials.¹

One feature of all of our DNMS experiments precludes any likelihood that rats were solving the task by discriminating the relative recency with which the sample and novel objects were handled. We have employed retention delays ranging from 15 sec to several minutes in...
all of our DNMS experiments. If a discrimination based on touch-produced odour cues supported DNMS performance, then the objects would have to remain distinguishable on this basis after delays lasting several seconds. In the discrimination experiment described above, the rats eventually learned to discriminate which object was touched most recently with a 4 sec delay between when S was last touched and when they were allowed to attempt the discrimination. However, their performance dropped to chance levels when the delay was increased to 15 sec, and it did not improve with further training with that delay. Presumably, the difference between the intensity of the experimenter’s odour that remained on the two objects became imperceptible to the rats within 15 sec. In contrast, we have repeatedly found that increasing the retention delay from 4 sec to 15 sec has little effect on rats’ DNMS performance, and they continue to perform well with delays of several minutes.2,3

Given the results of their probe trials, it is not surprising that Herremans et al didn’t bother to assess their rats’ DNMS accuracy at delays longer than 4 sec. But, without having done so, they have not replicated the DNMS testing procedures employed by Mumby et al, which involved post-acquisition training at progressively longer delays ranging from 15 to 600 sec, and the subsequent determination of each rat’s retention function with mixed-delay sessions. Herremans et al’s results with a 4 sec delay cannot explain how rats are able to solve the DNMS task at much longer delays.

The findings of Herremans et al, and those of our experimenter-odour-discrimination experiment described above demonstrate the importance of carefully controlling the odour cues to ensure that they do not confound the intended sensory-processing demands of object-memory tasks. Obvious precautions include experimenters washing their hands between test sessions and after placing a rat into the apparatus at the beginning of a session, minimizing the amount that they handle the objects, and avoiding systematic differences between choice stimuli in terms of how much or in what order they are handled. For tasks that involve both a sample-phase and a test-phase on each trial, such as matching- or nonmatching-to-sample, the possibility of an olfactory solution can be eliminated by using two identical objects as the sample of each trial—one serves as the sample on the sample phase, the other serves as the sample on the test phase, and all of the objects can be positioned prior to the beginning of the trial. We now conduct our DNMS testing this way and we have found that rats can perform at levels that are comparable to those achieved by rats tested with our original procedure.6

Although the rats in our experiments were not solving the DNMS task by discriminating the relative recency with which the sample and novel objects were handled, the results of Herremans et al’s probe trials are consistent with their conclusion that their rats were solving the task this way. However, it is worthwhile to consider some alternative possibilities, as this will help to highlight other potential pitfalls of administering DNMS and other object-memory tasks. Seemingly unimportant features associated with the layout of the test apparatus, the behaviour of the experimenter, or details of protocol can provide the means for rats to circumvent the intended cognitive demands of the DNMS task. For example, sounds that are associated with the placement of objects over the food wells could provide auditory cues indicating the location of the most recent object placement. If the apparatus is not elevated high enough from the floor, or if its walls and doors are not big enough, the movements made by the experimenter when positioning the objects over the two food wells could be distinguishable by the rats. A rat could solve the task by learning to go directly to where it hears or sees an object being placed. The results of Herremans et al’s probe trials are exactly what one would expect if their rats had inadvertently been trained to employ such a strategy.

Our rats were able to master the DNMS task when the order in which the sample and novel objects were placed into the goal areas was randomised across trials. Why were Herremans et al’s rats unable to do so? There are several possibilities. Unfortunately, we became aware of many factors that can prevent DNMS acquisition only after the original description of our DNMS paradigm.

The features of the objects that comprise the stimulus pool are important. We include only objects that we consider to be highly distinctive in terms of their visual features, including size, shape, colour, brightness and intrinsic patterns. It has been our impression that size is an important dimension; objects that are more than 7 cm in their longest axis work best, whereas objects that are smaller than this are almost impossible for rats to distinguish on the basis of visual features. We do not know the typical size dimensions of the objects used by Herremans et al, but a stimulus pool comprising mostly small objects would discourage a visual working memory strategy and perhaps encourage a discrimination involving touch-produced experimenter-odour cues.

The behaviour of the rat is also important. There are three problems that we encounter most frequently and that seem to preclude accurate DNMS performance: rats run at a pace that is either too slow or too fast, or they display side-biases (eg, always pick whichever object is on the left). The latter two appear together most of the time. Rats that run too slowly are prone to distraction during trials and during retention delays. Rats that run too fast will make frequent errors in their haste, and they might not take sufficient time to examine the sensory features of the sample rats at the beginning of a trial. Optimal DNMS performance by a group of rats requires adjustment of each rat’s daily food ration in accordance with their current running speed: rats that are running too slowly should receive slightly less food and rats that are running too quickly should receive slightly more. Slowing down a hasty rat is usually all that is needed to eliminate a side bias, but additional measures designed to shape the rat away from its bias might be necessary in some cases. A rat in one of our experiments typically receives 20 - 30 g of chow per day, depending on its running speed. In contrast, Herremans et al fed their rats only 13 g of chow per day. Our experience suggests that such extreme food deprivation would cause rats to run much too quickly, to develop side-biases like those reported by Herremans et al, and to fail to attain consistently high levels of DNMS performance.

The present commentary on the report by Herremans et al makes three general points: First, contrary to the conclusions of Herremans et al, the DNMS
procedures described by Mumby et al can be used to assess working-memory for objects in rats. Second, much care is needed to ensure that the testing conditions do not inadvertently provide and encourage unintended solutions to object-memory tasks. Third, several methodological flaws are capable by themselves of precluding accurate DNMS performance. Unfortunately, there are many things that can go wrong with nonautomated memory tasks, in which the experimenter plays such an active and ongoing role. The situation is familiar to that facing human neuropsychologists, who must take meticulous care to administer tests in ways that will avoid introducing unwarranted variance into the performance of a patient, or accidentally cueing the patient and thereby enabling them to circumvent the intended cognitive or behavioural demands of the tests. Experimenters who choose to administer nonautomated memory tasks to laboratory animals must remain alert for the development of subtle habits during monotonous and repetitive testing, and ensure that they don't inadvertently provide cues that enable the rat to circumvent the intended cognitive demands of the task. The difficulties are amplified when multiple experimenters are responsible for testing the same group of rats. In those situations, care must be taken to ensure that the experimenters' techniques are calibrated to match one another.

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References