RECOGNITION MEMORY IN RATS—I.
CONCEPTS AND CLASSIFICATION

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Abstract—Recognition is the process by which a subject is aware that a stimulus has been previously experienced. It requires that the characteristics of events are perceived, discriminated, identified and then compared (matched) against a memory of the characteristics of previously experienced events. Understanding recognition memory, its underlying neuronal mechanisms, its dysfunction and alleviation of the latter by putative cognition enhancing drugs is a major research target and has triggered a wealth of animal studies. One of the most widely used animals for this purpose is the rat, and it is the rat’s recognition memory which is the focus of this review. In this first part, concepts of recognition memory, stages of mnemonic processing and paradigms for the measurement of the rat’s recognition memory will be discussed. In two subsequent articles (parts II and III) we will focus on the neuronal mechanisms underlying recognition memory in rats. Three major points arise from the comparison of paradigms that have in the past been used to assess recognition memory in rats. First, it should be realized that some tasks which, at face value, can all be considered to measure recognition memory in rats, may not assess recognition memory at all but may, for example, be based on recall rather than recognition. Second, it is evident that different types of recognition memory can be distinguished and that tasks differ in the type of recognition memory taxed. Some paradigms, for example, measure familiarity, whereas others assess recency. Furthermore, paradigms differ as to whether spatial stimuli or items are employed. Third, different processes, ranging from stimulus–response learning to the formation of concepts, may be involved to varying extent in different tasks. These are important considerations and question the predictive validity of the results obtained from studies examining, for example, the effects of putative cognition enhancing drugs.

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1. INTRODUCTION

The ability to recognize stimuli—or knowledge of whether we have experienced something in the past—may help us to put present items more easily into context, such as to classify items as being associated with positive or negative experiences, or to enable us to use them as cues, a capacity with definite evolutionary relevance. A failure in recognition memory on the other hand has obvious impacts on daily living and will contribute to the problems encountered by many amnesic or demented people. Understanding recognition memory, its underlying neuronal mechanisms, conditions of its dysfunction and alleviation of the latter by putative cognition enhancing drugs are therefore major research targets which have lead to a wealth of animal studies. One of the most popular animals for this purpose is the rat, and it is the rat’s recognition memory which is the focus of this review.

We shall define recognition memory as neural process(es) by which a subject is aware that a stimulus has been previously experienced and recognition as the behavioural outcome of these processes. It requires that the perceived characteristics of events are discriminated, identified and compared (matched) against a memory of the characteristics of previously experienced events. It is this combination of processes and representations which characterizes what is frequently termed recognition memory. Accordingly, an essential feature inherent to recognition memory tasks must be an experimental design which allows a comparison between presented items (or events) and previously stored information. In empirical contexts, recognition memory tasks are based on the general principle of matching. Trials characteristically consist of three phases: a sample phase, a delay phase (or retention interval) and a choice (or comparison) phase. In the first, sample phase, a stimulus is presented and the subject has to store information about this stimulus and to retain this information over the delay phase for subsequent, successful performance in the choice phase. In this latter phase, choice stimuli are presented and the subject has to compare these stimuli against the previously stored information. Trials can be either discrete, i.e. present sample stimulus and previous choice stimuli may be different, or continuous, i.e. the previous comparison stimulus becomes the sample stimulus of the present trial. Within this framework, two basic rules for responding are possible: matching and non-matching. Under matching conditions, a subject is trained to respond to the choice stimulus that resembles the sample, whereas under non-matching conditions, a correct response is made by choosing the stimulus which has not been presented as the sample. The delay phase is interposed between sample and choice phases and consists of a time interval during which either no sample or choice stimuli at all are presented (as in the majority of paradigms) or where for the particular comparison irrelevant stimuli are displayed (as can be seen with lists of different sample stimuli). Selection of different delays allows variation of mnemonic load, i.e. the study of retention of information over time. This variation usually manifests itself in a progressive decline in accuracy with longer delays, resulting in a forgetting curve and the slope of this curve provides information about the mnemonic ability of the subject—it is generally considered that the steeper the slope, the more rapid the loss of information. However, it should be noted that a delay-dependent decline in accuracy is not necessarily related to mnemonic ability. Certain paradigms (delayed response tasks; see below) allow the use of mediating strategies, i.e. allow the animal to orientate itself already during the delay phase towards the subsequently correct comparison stimulus. It has been demonstrated that rats can use clearly identifiable mediating strategies which help the animal performing the task, and which disappear at longer delays (Chudasama and Muir, 1997).

This review starts with an overview of the concepts used within the studies of recognition memory. The stages of mnemonic processing involved in recognition memory will be discussed with emphasis on the distinction between mnemonic and non-mnemonic processes. Finally, presently used paradigms for the measurement of recognition memory will be explained and evaluated.

2. CONCEPTS OF RECOGNITION MEMORY

There is a bewildering variety of mnemonic processes which can be assessed in animals and humans. It is therefore useful for an understanding of recognition memory to develop a framework which puts this type of memory into context with other cognitive processes.

In a typical recognition memory task, two sets of information are required. First, in all but the spontaneous preference tasks information has to be remembered about a general rule as to how to respond. This information is stored in so-called reference memory and is recalled, not recognized.
Recall is the retrieval of information from a memory store, but does not rely on stimulus discrimination/identification or stimulus comparison.

Second, trial-specific information is required for accurate performance. Memory that is specific for a single trial has been defined by Olton et al. (1980) as working memory. According to this definition, recognition memory tasks also tax working memory. However, it is important to note that working memory as measured in a recognition memory task with its three phases (sample–delay–choice) differs from the working memory measured in tasks requiring a relational representation, such as in many maze paradigms (Eichenbaum et al., 1994). Essentially, all stimuli are available in a typical maze working memory task and it is not necessarily a specific stimulus but the previous episode of entering a maze arm, i.e. the response, that will be remembered. This can be recalled and does not necessarily involve recognition. In a recognition memory task, the presentation of a stimulus is, however, terminated at the end of the sample phase. Therefore, it is the sample stimulus itself which has to be remembered throughout the delay phase, and the subject has to match between choice stimuli and a memory trace of that sample stimulus at a later stage. It is this trial-specific component which is of primary interest when we look at recognition memory.

Related to the distinction between the remembrance of a response and the remembrance of the stimulus itself is the distinction between delayed response and delayed comparison paradigms. This is explained below.

2.1. Delayed Response and Delayed Comparison

As outlined above, a typical recognition memory paradigm consists of presentation of a sample stimulus, followed by a retention interval and, subsequently, the presentation of choice stimuli. In the vast majority of delayed comparison tasks for rats, one sample stimulus is presented out of two (or more) possible sample stimuli, the subject has to retain information about the characteristics of the sample stimulus over the delay period, and then the choice stimuli have to be compared as to whether they match or non-match to the sample. Only then can the subject decide whether or not, or where, to respond. The underlying process is a delayed conditional discrimination.

A sample stimulus is also presented in delayed responding, but all information necessary for correct responding is given at the sample stage (delayed alternation, for example, is a delayed response task; Fig. 1). In contrast to delayed comparison paradigms, it is the essential feature that the subject is able to correctly predict which spatial position to respond to in delayed response tasks.

There are at least three points highlighting the relevance of this distinction. First of all, it is possible that the information remembered in delayed response tasks is recalled rather than recognized. Given that a subject retains information about the correct response rather than information about the sample stimulus in delayed response paradigms, the underlying process is delayed recall and not delayed recognition. In keeping with Eichenbaum’s et al. (1994) distinction, both delayed recall and delayed recognition involve working memory but are obviously different.

Second, it is possible that the memory load in delayed response paradigms is minimized if a mediating strategy is employed by the subject, i.e. that the subject orients to the response when the sample stimulus is presented and then adopts a strategy such as to wait in front of a response lever until the end of the delay (for example, Herremans et al., 1994; Stanhope et al., 1995; Herremans et al., 1996; Pontecorvo et al., 1996; Chudasama and Muir, 1997). Since the subject is unable to correctly predict where to respond in delayed comparison paradigms, neither overt mediating strategies nor delayed recall can be used in the latter type of tasks (Pontecorvo et al., 1996).

Third, sample stimuli employed in delayed comparison paradigms are usually visual, auditory or olfactory in nature, or are complex, three-dimensional objects. In any case, the sample stimulus is non-spatial (note that the response may well be spatial in nature). Delayed response, however, may already tax spatial memory at the sample stage if the response and not the sample is remembered. In fact, even the sample stimuli employed in most delayed response tasks are spatial in nature, for example, if a discrimination between a left and a right lever, or a left and a right stimulus light is required. Thus, it may be suggested that memory for items (objects/non-spatial samples) is more readily taxed in delayed comparison procedures, whereas spatial memory is required to a greater extent in delayed response tasks. This leads us to the next distinction which has been of major importance in the discussion of recognition memory in a wide variety of species, namely that between item memory and spatial memory.

2.2. Memory for Items and Memory for Spatial Context

Ungerleider and Mishkin (1982) suggested a dichotomy in the non-human primate temporal lobe structures mediating object and spatial memory and, more recently, Goldman-Rakic (1988) distinguished different prefrontal areas as being involved in the processing of spatial information and of information about object characteristics in primates. A similar dichotomy in the neural systems mediating recognition memory has been suggested in humans (Courtney et al., 1996; McCarthy et al., 1996), and Kolb et al. (1994) hypothesized that this dissociation can also be made in the rat. It is therefore plausible that recognition memory tasks which rely differently on spatial memory processes activate different parts of the rat’s brain.

Gaffan (1992) developed a further distinction by dissociating between memory for specific objects and memory for whole scenes, a dichotomy which can be regarded as being related to the previous one, but which goes beyond a simple spatial/item differentiation in that scene-specific memory can involve an item placed in a particular scene/context, for example, the experimental task. Gaffan demon-
Fig. 1. Delayed alternation (DA). A typical session starts with a forced sample phase, in which the animal is forced (start situation) to choose (bold arrow) one of usually two stimuli, to press the only inserted lever of two retractable levers or to enter the only accessible arm of a Y-maze or T-maze, either sample A on the left position, or sample B on the right position (the shaded sample indicates unavailability during the forced sample phase). After a delay, trial 1 continues from a start situation with the animal's delayed response. The animal is now free to choose either stimulus/position A or stimulus/position B. Subsequent presentation of reinforcement is indicated by "+", while absence of reinforcement is indicated by "−". From this point onwards the animal's choice on the present trial will become the sample for the following trial, thus each trial has a combined choice/sample phase. If the animal chooses the stimulus/position, which was responded to during the previous trial it is considered an error and reinforcement will not follow ("−"). If the animal chooses the alternative stimulus/position, to which previously was not responded, then it is considered a correct response and reinforcement follows ("+"). This reinforcement protocol is maintained throughout all following trials, so that an adequately responding animal will alternate between the two possible stimuli/positions. Note that in this paradigm the delay period coincides with the intertrial interval, a feature that constitutes the continuous character of this task. The figure shows an example of a possible response sequence (arrows), starting from a random forced sample, i.e. sample position A, with three examples of correct responses (trials 1, 2 and 4) and one example of an incorrect response (trial 3).
strated that different parts of the primate temporal lobes are involved in the mediation of these two types of memory (Gaffan, 1992, 1994). An associated concept in the human literature is the distinction between recognition of specific stimuli as opposed to recognition of stimuli within a specific context (Mandler, 1980).

To what extent this dichotomy between item and scene memory will contribute to work on recognition memory in rats is unclear at present. However, it is noteworthy that two forms of conditioning and conditioning to explicit cues have been distinguished, namely contextual conditioning and conditioning to explicit cues (Maes and Vossen, 1993a,b), and that these two forms of conditioning are probably mediated by different brain areas in rats (Selden et al., 1991). This may suggest that Gaffan’s distinction may also be of relevance for an understanding of mnemonic processes in this species.

A more detailed account of the brain structures involved in the mediation of these processes is given in the second part of this review (Steckler et al., 1998). Here we note that recognition memory subsumes at least two qualitatively different memory processes which appear to involve different areas of the brain.

We can take the distinction of recognition memory further by considering the number of exposures a subject had to a given stimulus. As a consequence we have to discuss the issues of familiarity/novelty as well as a time element (recency).

2.3. Memory for Familiarity and Memory for Recency

Recognition has often been defined as the ability to discriminate stimuli of different familiarity, i.e. the discrimination is between familiar and novel stimuli, and large sets of stimuli have been used in order to minimize repetitive presentation of stimuli in paradigms taxing memory for familiarity. Many recognition memory tasks, however, require a discrimination between stimuli which are of similar familiarity, but which differ in recency of presentation. Usually, limited sets of stimuli are employed in the latter tasks.

There are at least three points which require consideration in the comparison of tasks employing different set sizes of stimuli. First, information acquired on a previous trial may influence the acquisition of information during an ongoing trial, a process called proactive interference (for a more detailed account see section on “Proactive interference”, below). Proactive interference could occur when the sample from the previous trial differs from the sample on the current trial. Therefore, it follows that proactive interference will be of greater influence in studies testing familiarity than in those examining recency.

Second, it can be suggested that repetitive presentation of stimuli involves another type of discrimination learning than that if the discriminanda are new on every trial (trial-unique). Repeated stimulus presentation may involve simultaneous discrimination such as in delayed response paradigms, where the discrimination may be between two responses, as outlined above, or it may involve a delayed conditional discrimination, which is possible in both delayed response and delayed comparison tasks. Furthermore, it is possible that concurrent discrimination, i.e. the discrimination of sequentially presented stimuli, is required if lists of a limited set of stimuli are presented because animals also have to distinguish between the different sample stimuli displayed prior to comparison. All these different types of discrimination can be performed by rote without requiring the subject to form a concept. In contrast, presentation of trial unique stimuli is based on one-trial learning and requires concept formation, i.e. unitary events are not only remembered but integrated into a larger body of knowledge which incorporates the general rule of matching.

Here, an essential criterion is that the stimuli must be new on critical test trials (Thomas, 1996).

Third, discrimination of recency may tax temporal context memory where a temporal order judgement is required. Temporal context memory is not required in studies of familiarity where the judgement is between known and unknown stimuli.

The relevance of the distinction between familiarity and recency has been shown in humans (Shaw and Aggleton, 1995) and can, for example, be illustrated for the rat in a study by Zhu et al. (1995), measuring single neurone recordings in rats exposed to repeated presentation of objects of different familiarity. They showed that neurones encoded both the relative familiarity of a stimulus and the information that it had been seen recently. Moreover, it was possible to identify “recency neurones” that signalled recency but not familiarity of stimuli, and “familiarity neurones” that responded to familiarity but not recency.

Rawlins et al. (1993) demonstrated that memory for familiarity and memory for recency are differentially susceptible to lesions of the brain. In this study, rats with lesions of the fornix or hippocampus were tested in delayed matching to sample (DMTS) in an apparatus comprising of a start area which expanded into two goal areas; goal boxes contained complex objects or differed in paint finish and texture. Lesioned animals showed an impairment only when the stimuli to be remembered were used repeatedly within sessions, which could be interpreted as an impairment in recency discrimination. However, this impairment was greatest with plain goal boxes. Therefore, it was suggested more recently (Cassaday and Rawlins, 1995) that the Rawlins et al. (1993) results may have been confounded by inadvertent introduction of differences in spatial features between small and large sets of stimuli used. Thus, the lesions may have caused an impairment in spatial rather than in recency discrimination; Mumby (1995) also reported that rats had less difficulty in discriminating objects more than 7 cm in their longest axis. However, the electrophysiological data by Zhu et al. (1995) clearly indicate that there may be qualitative differences between memory for familiarity and memory for recency in that different neuronal systems may mediate these two processes.

Alternatively, it is possible that tasks assessing memory for familiarity and memory for recency affect the capacity of the neuronal systems involved.
in different ways since they differ in the number of stimuli employed and hence in the degree of proactive interference involved.

### 2.4. Recognition Involves Different Levels of Cognitive Abilities

Obviously, discrimination between stimuli is a process crucial in recognition memory. However, as already pointed out, different types of discrimination could be involved, depending on, for example, the number and originality of the stimuli used, and it is important to note that stimulus discriminations can be at different levels of cognitive abilities. Such levels of cognitive abilities which appear to be pertinent for our discussion are stimulus–response learning, chaining, i.e. the learning of a series of stimulus–response units, concurrent discrimination learning, i.e. learning stimulus-response units in parallel, absolute and relative class concepts, and relational class concepts (see Thomas, 1996 for development of a hierarchy of learning abilities). One obvious example has been given earlier in the discussion of memory for familiarity and memory for recency, where we pointed out that repetitive presentation of stimulus and reinforcement involves levels up to concurrent discrimination learning, whereas the formation of concepts is required if trial-unique stimuli are used as the rat has no possibility to learn the relationship between two stimuli, but must develop a concept of matching in the latter case (Thomas, 1996). Furthermore, it can be suggested that a task based on the minimum set of two stimuli differs from tasks involving sets of several (repeatedly presented) stimuli in that the latter involve chaining and/or concurrent discrimination learning.

In addition, animals have to distinguish between sample and comparison phases. If sample and comparison stimuli are presented in different spatial locations, such as the sample in the centre and the comparison left and right from this position, animals may use spatial cues to make this distinction. This may be of importance, for example, in hippocampally lesioned rats, where the lesion could interfere with a spatial discrimination.

There is much debate whether rats can form concepts at all (see Thomas, 1996 for discussion). However, results from studies testing memory for familiarity with trial-unique stimuli support the hypothesis that concept formation is possible in rats. Shaw and Aggleton (1993), for example, trained rats in a Y-maze delayed continuous non-matching to sample (CNMTS) paradigm where animals had first to explore a sample object placed in a start arm of the maze and then had to select a goal arm that contained an unfamiliar three-dimensional junk object over another goal arm that contained an object similar to the sample object. Once animals were trained on the task the authors introduced a new set of trial-unique objects. Animals performed considerably better than chance, suggesting that they were able to form a concept.

From this we can conclude that the ability to recognize is not restricted to a certain level of learning ability. This is of importance since different recognition memory tasks may differ in task difficulty.

### Table 1. Procedures used for the assessment of recognition memory in the rat

<table>
<thead>
<tr>
<th>Task</th>
<th>Classification</th>
<th>Set size</th>
<th>Stimulus modality</th>
<th>Choice</th>
<th>Number of comparisons</th>
<th>Test of Discrimination</th>
<th>Recency or familiarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>DA</td>
<td>Delayed response</td>
<td>Small 2</td>
<td>Forced</td>
<td>Forced</td>
<td>2-3</td>
<td>Continuous</td>
<td>Delayed conditional or simple</td>
</tr>
<tr>
<td>DMTP/DNMTP</td>
<td>Delayed comparison</td>
<td>Small 2</td>
<td>Forced</td>
<td>Forced</td>
<td>2</td>
<td>Discrete</td>
<td>Delayed conditional or simple</td>
</tr>
<tr>
<td>DMTS/DNMTS</td>
<td>Delayed comparison</td>
<td>Small- large (pseudo-trial-unique)</td>
<td>Non-spatial</td>
<td>Forced</td>
<td>1-2</td>
<td>Continuous</td>
<td>Delayed conditional or simple</td>
</tr>
<tr>
<td>CNMTS</td>
<td>Delayed comparison</td>
<td>Small medium 2-16</td>
<td>Non-spatiald</td>
<td>Forced</td>
<td>Continuous</td>
<td>Delayed conditional or simple</td>
<td></td>
</tr>
<tr>
<td>DPC</td>
<td>Delayed comparison</td>
<td>Small 2</td>
<td>Non-spatial</td>
<td>Go/no-go</td>
<td>1 or 2</td>
<td>Continuous</td>
<td>Delayed conditional or simple</td>
</tr>
<tr>
<td>DCC</td>
<td>Delayed comparison</td>
<td>Small medium 2-16</td>
<td>Non-spatiald</td>
<td>Forced</td>
<td>1 or 2</td>
<td>Continuous</td>
<td>Delayed conditional or simple</td>
</tr>
</tbody>
</table>

Note that delayed conditional discrimination (DCD) is not included as it is more likely to measure delayed recall.

aNumber of comparisons refers to the number of choice stimuli presented per trial.
bLarge sets of stimuli usually comprise of 50–300 stimulus pairs in D(N)MTS, CNMTS; larger sets of stimuli used in DCC comprised up to 16 different stimuli.
cMaze tasks allow place, cue or egocentric navigation, whereas operant chambers are likely to restrict responding to cue or egocentric strategies.
dThese tasks are not purely non-spatial as the response involves a spatial component.

DA, delayed alternation; DMTP, delayed matching to position; DNMTP, delayed non-matching to position; DMTS, delayed matching to sample; DNMTS, delayed non-matching to sample; CNMTS, continuous non-matching to sample; DPC, delayed paired comparison; DCC, delayed continuous comparison.
and tax different levels of cognitive abilities (see also Table 1, which provides a summary of the differences of various paradigms used to measure recognition memory in rats).

Not only are these distinctions important for an understanding of the rat’s recognition memory, but also of practical relevance for reasons of interspecies comparability. Rats, for example, are frequently used to test drug or lesion effects on recognition memory, primarily in delayed response paradigms with small sets (most often two) of stimuli. Many of these studies are aimed at modelling the recognition deficits seen in man—deficits which are, however, primarily assessed in delayed comparison tasks which frequently comprise large stimulus sets. Moreover, different levels of cognitive abilities are required in different recognition memory tasks. Thus, rat and human studies may differ in the processes taxed in the paradigms employed. This in turn may yield different outcomes, for example, in psychopharmacological experiments, and therefore is likely to bear problems for inter-species comparison (see Steckler and Muir, 1996, for an overview).

3. STAGES OF MNEMONIC PROCESSING INVOLVED IN RECOGNITION MEMORY

From the previous section it is evident that recognition memory can involve cognitive processes at different levels, ranging from stimulus-response learning to the use of class concepts. It will be assumed that these cognitive processes are hierarchically organized for the sake of being able to classify these processes, albeit at the cost of being oversimplistic. Moreover, at each of these levels, further dissociations can be made. First, a subject has to acquire/encode information about a sample stimulus. Second, this information needs to be maintained over the delay and, third, be retrieved after the delay to allow comparison. Furthermore, performance may depend on the time interval between presentation of the sample and the comparison stimuli and may be facilitated by rehearsal—although it should be noted that it is a matter of debate whether rats can rehearse at all, i.e. actively evoke or maintain a memory trace. Moreover, information processing will be affected by interference and motivational factors. There is extensive literature dealing with these topics, but most studies employ species other than rats. However, considering the task demands and mnemonic processes involved it seems reasonable to suggest that the same processes which affect recognition memory performance in other species are of importance for recognition memory performance in rats.

3.1. Acquisition/Encoding vs Storage and Retrieval

Acquisition/encoding has been assumed to be positively related to the duration of exposure to a sample stimulus, i.e. increasing exposure time improves acquisition/encoding and vice versa, but should have no direct effect on retention of information (Van Hest and Steckler, 1996). Indeed, rats given a longer sample period duration improve their rate of acquisition and subsequent performance in non-matching to sample tasks (Beck and Kalyanchuk, 1992). Alterations in sample exposure time have also been used to examine drug effects on acquisition/encoding in pigeons (Sahgal et al., 1980), but such detailed drug- or lesion-studies have not been done yet with rats.

Whether rats directly match the perceived characteristics of a comparison stimulus with those remembered from a sample stimulus or rather use a generalized matching concept, transforming the information about the sample into a code which is only arbitrarily related, is at present unclear. However, in a recent study by Savage and Langlais (1995), it has been shown that different outcome procedures, where the correct choice of each comparison stimulus is followed by a unique outcome, enhances accuracy in rats. This suggests that rats code the outcome as part of their representation of the correct choice, i.e. that rats code the comparison stimulus and its outcome prospectively (see also Roitblat, 1993, arguing this point for pigeons).

3.2. Retention

The retention interval between sample presentation and choice can be regarded as the most important procedural variable in tasks measuring recognition memory, as performance is a negatively accelerated function of the delay duration between the presentation of the information at the beginning of the trial and the opportunity to respond at the end of the trial (Dunnett et al., 1988; Van Haaren and Van Hest, 1989), i.e. the rate of forgetting increases with increasing length of the delay. It can be assumed that load on retention is least at shortest delays and increases with increasing delay duration.

Thus, two cognitive functions will be taxed by simultaneous presentation of sample and comparison stimuli: reference memory, i.e. memory about the rule about how to respond, and mere discriminative abilities. By definition, recognition memory, however, will not be involved under this condition. Simultaneous matching can be scheduled in delayed (non)matching to sample [D(N)MTS] and delayed paired comparison (DPC; these tasks will be described in detail under Section 5) and this possibility represents one of the great advances of these types of tasks over other delayed matching procedures.

Successive, but immediate presentation of sample and comparison stimuli, which is called a zero-second delay condition imposes a minimal load on retention, but it can be suggested that encoding and retrieval processes gain greater importance. The inability to test animals under zero-second delay conditions is a problem inherent to the non- or semi-automated tasks as well as to all continuous delayed (non-) matching paradigms described below. The inability to assess performance under zero-second delay conditions, however, renders interpretation of data problematic; it can be expected that a manipulation which specifically affects retention should not alter performance at zero-delay, whereas changes in non-specific factors (discriminative ability, but also motivation and/or motor function) can be evoked if performance is affected at zero-delay. Thus, the in-
formation gained from simultaneous matching and zero-second delay trials is very useful in data interpretation.

Increasing the retention interval is generally accepted to impose increasing mnemonic load. This will result in a forgetting curve against which other manipulations can be compared. Two points, however, need consideration: First, Ringo (1988, 1992) raised the issue that the most frequently used performance measure, percentage correct responses, often shows a ceiling effect in matching paradigms. This may then be interpreted as a delay-dependent treatment effect. The problem of ceiling effects also implies that experimental groups need to be matched over all delays used, as matching groups at short delays only may result in inappropriately matched groups which differ at longer delays.

Secondly, it is possible that rats exhibit different response strategies at short (0.5 sec) delays, but employ spatial stimuli at longer (100 sec) delays, and Chudasama and Muir (1997) demonstrated that rats used mediating behaviours at short delays, which disappeared at longer delays in an operant DNMTP task. If that is the case, then delay-dependent drug or lesion effects could simply reflect different vulnerabilities of different cognitive processes. Despite these caveats, it is concluded that it is difficult to interpret results from tasks lacking delay intervals in terms of effects on recognition memory.

3.3. Proactive Interference

Recognition memory is susceptible to proactive interference. Two different forms of proactive interference effects have been distinguished (Edhouse and White, 1988; Roitblat and Harley, 1988; Roitblat, 1993; White et al., 1996). We have discussed the first type of proactive interference, which relates to the degree of intrusions from one trial to the next. In other words, the information acquired on a previous trial influences the acquisition of information during an ongoing trial. This type of proactive interference occurs when the sample from the previous trial differs from the sample on the current trial. In rats, the influence of the events on the previous trial declines with increasing ITI duration.

The second type of proactive interference depends on the duration of the intertrial interval (ITI) and manifests as an overall improvement of performance with longer ITI’s (Dunnett and Martel, 1990; Dunnett et al., 1990; Herremans et al., 1994; Roitblat and Harley, 1988). This may result from altered information processing (Roitblat, 1993; Roitblat and Harley, 1988) or attention to the sample (White et al., 1996).

Discrete trial procedures allow variation of the ITI duration as well as the parametric variation of the pattern of stimulus presentation, i.e. the assessment of both forms of interferences. Naturally, the assessment of proactive interference based on variation of the ITI duration is impossible in continuous trial procedures as the ITI is equivalent with the delay interval. However, it remains possible to assess the influence of the first type of proactive interference (Pontecorvo, 1983).

3.4. Retroactive Interference

Retroactive interference is another source influencing mnemonic performance. Here, newly acquired information or intervening activity affects retrieval of memories or more directly the stored information itself, acquired earlier in time. Consideration of the effects of retroactive interference in tasks measuring recognition memory is important as most delayed response tasks are characterized by a requirement for the rat to show controlled behaviour (for example, nose-poke, lever press) during the delay period, which is crucial for the prevention or at least reduction of mediating strategies. The number of such responses normally increases with increasing delays. Moise (1970), for example, trained monkeys to press a key during the delay interval in a delayed matching task. Accuracy was already disrupted when a single press was required, and disruption of performance increased with increasing response requirement. There is no comparative study with rats, but it can be assumed that retroactive interference induced by the response requirement during the retention interval contributes to the slope of the forgetting curve in these paradigms.

Retroactive interference has been studied in rats, but apparently not in operant tasks measuring recognition memory. Jarrard (1975) assessed the role of retroactive interference in a Y-maze, and delayed the alternation task by forcing the animals to run in an activity wheel during delays ranging from 0 to 4 min. Interpolated activity did not affect retrieval of information in controls, but affected performance in rats with hippocampal lesions. Beatty and Shavalia (1980), using an eight-arm radial maze DNMTP procedure, showed that interpolation of another 8-arm radial maze task during a long delay of 4 hr did not affect subsequent choice performance, suggesting again that retroactive interference does not affect performance in normal rats. It is, of course, possible that processes other than recognition memory are activated in animals performing these tasks. However, the important point derived from Jarrard’s (1975) study is that treatment-induced group differences are not necessarily based on impaired recognition memory, but could be due to higher susceptibility to interference.

4. DISTINGUISHING MNEMONIC FROM NON-MNEMONIC PROCESSES

Several procedural parameters influencing performance have been identified and recently reviewed (Van Hest and Steckler, 1996). Suffice to say here that the characteristics of the sample stimuli, sample response requirements, the duration of the delay
interval, reinforcement contingencies and the inter-trial interval duration will all affect recognition memory performance.

Percentage correct responses over consecutive delays is the standard accuracy measure in delayed response/delayed comparison paradigms, and mnemonic and non-mnemonic effects of a manipulation can be differentiated depending on whether group differences are inversely related to length of delay. However, there are problems with ceiling effects in studies where percentage correct responses approaches 100% at short delays. A simple solution to the problem would be to train animals on a paradigm that avoids ceiling effects, for example, in a task where animals reach a maximum of about 80% correct responses at short delays. This, however, is difficult to achieve if stable performance is required over longer delays. Another way of reducing the problem is to use an arcane transformation of the data or by calculating indices derived from the mathematical methods of signal detection theory (SDT), such as the accuracy index SI, which magnify values at high performance level (Ringo, 1988; 1992; Marston, 1996).

Furthermore, it is possible that restriction of data analysis to the percentage correct measure is misleading due to changes in motivation or motor function. For example, it can be suggested that under extreme conditions accuracy is reduced to the point that animals revert to a response strategy as being the optimal solution. It is therefore important to evaluate whether an experimental manipulation really affects accuracy or motivational and/or motor processes, and this distinction is possible by using the methods of SDT (Heise and Milar, 1984; Sahgal, 1987; Pontecorvo and Cissold, 1993; Marston et al., 1993; Marston, 1996; Pontecorvo et al., 1996; White et al., 1996).

The majority of recognition memory paradigms are two-choice tasks. SDT in turn can be easily applied to two-choice tasks. In order to use the terminology of SDT, we have to arbitrarily define half of the samples as “signal”, and the other half as “noise”. A correct response to the “signal” (correct matching or non-matching) will be defined as a “hit”, an incorrect response to the “noise” will be defined as a “false alarm”. From the probabilities of “hits” and “false alarms” (“P(hit)” and “P(false alarm)”) it is possible to calculate indices of accuracy and bias (Sahgal, 1987; Marston, 1996). A detailed discussion of the different signal detection measures would be beyond the scope of this review. It should be noted, however, that this option to differentiate accuracy from bias clearly contributes to the strength of tasks measuring recognition memory in animals.

5. PARADIGMS FOR THE MEASUREMENT OF RECOGNITION MEMORY

There is a multitude of tasks used to assess recognition memory in rodents, ranging from maze paradigms to operantly controlled tasks, from tasks employing spatial stimuli to those based on non-spatial, complex visual, auditory or olfactory information. A distinction useful for characterization of these tasks is that between delayed response and delayed comparison procedures (Pontecorvo et al., 1996; see above), and this will be the basis for discussion of the various paradigms.

5.1. Delayed Response Paradigms

5.1.1. Spatial Delayed Alternation (DA)

DA paradigms are two-choice, continuous non-matching paradigms. They could be solved either by a delayed conditional discrimination at the time of choice or by a simple recency discrimination at the time of sample presentation.

A session starts with a forced sample phase, which could be the presentation of the left or right arm in a Y- or a T-maze (Heise and Milar, 1984; Olton and Markowska, 1993) or the presentation of the left or right lever in an operant chamber equipped with retractable levers (Heise and Milar, 1984), thus forcing the animal to make a distinct response (Fig. 1). Following a delay, the second trial starts in which the comparison stimuli—both maze arms or both levers—are presented and the animal has to choose the stimulus not presented during the forced sample phase. Subsequently, the task becomes a continuous delayed response paradigm in that the stimulus chosen during the previous trial becomes the sample stimulus of the new trial, i.e. the rat alternates between the two stimuli. All these tasks are spatial in nature.

Non-spatial, item DA has also been successfully used with rats, involving a go/no-go procedure (Peinado-Manzano and Pozo-Garcia, 1996). Here, each trial consisted of a sample phase, during which a lever was inserted into an operant chamber; the lever was retracted and, following a delay, presented again. Animals were trained to alternate between response contingencies, i.e. had to learn that they had to press the lever following the delay during half of the trials, but that they had to withhold responding during the other half.

It is, however, doubtful whether DA is of use for the assessment of recognition memory in the rat as the mechanisms which govern the response are not clearly defined, i.e. it is unclear as to whether it is stimulus recognition (for example, according to the rule “choose the least recently pressed lever”) or the alternation rule itself which controls behaviour. In the latter case it would not even be necessary to discriminate between stimuli, but the animal could use egocentric strategies (i.e. respond left then right then left then right, etc.).

5.1.2. Delayed Matching to Position (DMTP) / Delayed Non-matching to Position (DNMTP)

These discrete-trial procedures have been successfully employed in mazes, but are most frequently scheduled in operant chambers. They are also based on comparison of spatial stimuli and at least DNMTP can be seen as a paired event variant of the DA paradigm. Again, different spatial systems may be taxed with these tasks. Aggleton et al. (1995), for example, showed that lesions of the cingulum bundle did not impair operant DNMTP but
disrupted T-maze DNMTPT, while the reverse pattern of deficits was found following prelimbic cortex lesions (Aggleton et al., 1995). These dissociations suggest that the two tasks tax different types of memory and that the cingulum bundle carries information of only one of these types. What are the differences between a T-maze and an operant chamber? In the maze there is a multitude of distal spatial stimuli, distributed throughout the testing room, which can be used by the rat for orientation and favour allocentric, spatial strategies. In the operant chamber, on the other hand, the number of signals is restricted in the majority of studies, and signals are located within the test apparatus itself (for example, two levers, and a transparent side door), thus favouring cue strategies, i.e. orientation to a restricted set of stimuli, or even egocentric, praxis strategies, for example, by using a mediating strategy. Indeed, it has been shown that the side door provides a salient cue that aids DNMTPT performance (Ennaceur, personal communication). The relative paucity of salient cues in the operant chamber may be one of the reasons why rats can remember a sample (maze arms or operant manipulanda, respectively) over much longer delays (over hours) in maze-based DNMTPT when compared to operant DNMTPT, which, much shorter delays (usually less than 1 min) result in chance performance (see Pontecorvo et al., 1996, for a discussion of advantages and disadvantages of these two basic types of apparatuses).

Operant chambers are often equipped with two retractable levers which serve as spatial stimulus and operand, although alternative stimuli, such as stimulus lights and operand, for example, holes for nose-poking, have been used to schedule DNMTPT (Etherington et al., 1987; Robinson and Crawley, 1993; Gutnikov et al., 1994). During the sample phase either the left or the right lever is presented, the animal has to respond to the lever, and the lever is retracted. Following a delay, both levers emerge (choice phase). In DNMTPT (Fig. 2), the animal is required to press the lever that had been presented before, in DNMTPT the rat has to press the alternative lever (Dunnnett, 1985, 1993).

In the T- or Y-maze DNMTPT, there is a forced sample phase with either the left or right maze arm being blocked and then, following a delay, a choice phase with both maze arms being accessible (see Olton and Markowska, 1993, for an overview, and Dellu et al., 1992, describing a version of the task based on spontaneous exploration). Examples for DNMTPT scheduled in mazes also exist, employing different types of mazes and reinforcement; the latter paradigms have been, however, only infrequently reported in the literature (Roitblat and Harley, 1988; Bresnahan et al., 1992).

DNMTPT can also be scheduled in more complex mazes such as the eight-arm radial maze. Here, all eight arms are initially baited with food and the animal is allowed to visit four of the eight arms. Following a delay, correct responding consists of choosing those maze arms which have not been visited before and hence are still baited (see Olton and Markowska, 1993, for an overview). Although the number of choices is increased in the eight-arm radial maze task, this does not reflect an increase in stimulus set, which is defined by the number of different, concurrently presented groups of stimuli and, as outlined earlier, will determine to what degree recency and familiarity processes are involved. Rather, increasing the number of choices within a trial, i.e. simultaneous instead of concurrent presentation of stimuli, adds to the complexity of the response requirement. An increase in set size, albeit small, could be achieved by blockade of six of the eight arms, such that each trial consists of forced sampling in one arm, followed by choice between two arms only (thereby being comparable to the T-maze procedure). On the next trial, another set of two arms could be used, etc. Then, it can be argued, chaining or even concurrent discrimination would be involved and a different mnemonic level would be taxed. However, this assumes that the rat discriminates each maze arm on the basis of extramaze cues, i.e. features of the room serve as salient stimuli, rendering each arm of the maze unique. If the maze arm discrimination is based on their position relative to each other (i.e. left vs right) then it is not relevant which arms are employed as the discrimination may always be between two stimuli (left vs right maze arm), irrespective of their relative spatial location.

The same logic applies for operant paradigms: Gutnikov et al. (1994), for example, employed a five-choice operant chamber and presented different pairs of operand (illuminated holes). Again, the underlying assumption has to be that each illuminated hole is treated as a single stimulus. As soon as we assume that the light per se serves as the stimulus, irrespective of the hole in which it is presented, set size will be reduced to two stimuli (left vs right) presented in different locations, thereby increasing task complexity not set size.

Nevertheless, increasing task complexity per se may be of great advantage in operant DNMTPT since it reduces the likelihood of ceiling effects which represent a major problem in these tasks, as discussed above. Another strategy where it is clear that it is task complexity and not set size which is altered is the presentation of more than two comparison stimuli. Gutnikov et al. (1994), for example, increased task complexity by presentation of three illuminated holes during the comparison phase and animals were required to choose the matching hole out of three comparison stimuli instead out of only two. This design enhances task complexity without affecting set size. A similar increase in task complexity can be introduced by using three instead of only two retractable levers (Miyamoto et al., 1995), and a similar design could easily be adopted in maze tasks.

However, despite its popularity, D(N)MTPT has some disadvantages as a task of recognition memory, as these paradigms have been criticised for the fact that the to-be-remembered stimulus in these procedures is not clearly defined, since it could be the right or left lever/maze arm or, alternatively, the right or left response itself (Pontecorvo and Clissold, 1993; Pontecorvo et al., 1996). If it is the operandum or the maze arm which is remembered, these paradigms are, by definition, recognition memory tasks, since the to-be-remembered stimulus is
Fig. 2. Delayed matching to position (DMTP). A trial in DMTP begins with a sample phase in which one of a small set of stimuli, usually either sample A (left position) or sample B (right position), is presented. From this start situation the rat has to respond toward the presented sample (for example, sample A in trial 1; shaded samples indicate unavailability, such as a retracted lever) which initiates a delay. After this delay the choice phase starts with the presentation of both stimulus/position A and stimulus/position B followed by the delayed response of the animal. In DMTP the rat is rewarded for responding to the same stimulus/position as the sample/position that was presented during the sample phase. Presentation of reinforcement is indicated by “+”, while absence of reinforcement is indicated by “−”. After an intertrial interval the sample phase of the next trial is initiated with a randomly determined next sample/position. This discrete protocol is maintained throughout all following trials. The figure shows two examples of correct responses (trials 1 and 3) and three examples of an incorrect response (trials 2, 4 and 5). In contrast, in delayed non-matching to position (DNMTP) the animal is rewarded if its response is not towards the same stimulus/position as the sample/position that was presented during the sample phase (incorrect response), but towards the stimulus/position that does not match the sample/position presented during the sample phase (correct response). In the example shown reinforcement (“+” and “−”) would be reversed in case of DNMTP.
Fig. 3. Delayed conditional discrimination (DCD). During the sample phase of a delayed conditional discrimination task the animal is exposed to only one of several, usually two, possible samples (for example, two tones), either sample A or sample B (shaded samples are not presented during a given sample phase). The trial then automatically continues with a delay, after which the choice phase begins. For each sample a corresponding operandum is presented, choice A and choice B (for example, two levers). The rat is rewarded for responding to the choice which corresponds to the sample that was presented during the sample phase. Subsequent presentation of reinforcement is indicated by “+”, while absence of reinforcement is indicated by “–”. After an intertrial interval the sample phase of the next trial is initiated with a randomly determined next sample. This protocol is maintained throughout all following trials. Trials 1, 3 and 5 show correct responses, while trials 2 and 4 illustrate incorrect responses.
present at time of choice and can be recognized. However, as has been outlined before (see Section 2.1), the task reflects recall rather than recognition if it is the response which is remembered, since the animal has to retrieve the information about the previous sample response. It may therefore be more accurate to term this task a working memory paradigm rather than a recognition memory procedure.

5.1.3. Delayed Conditional Discrimination (DCD)

The to-be-remembered stimulus in DCD tasks is clearly defined, and, most frequently, rats are trained to discriminate between two auditory stimuli (high frequency vs low frequency tone; Kirk et al., 1988; Herremans et al., 1994), but discriminations between intensity of illumination (Andrews et al., 1992) or duration of visual stimulus presentation (Santi and Weise, 1995) have also been employed.

In DCD, animals are tested in operant chambers, the discriminative stimulus is presented as the sample and, following a delay, levers are inserted. The sample has to be remembered over the delay period and the response site has to be chosen contingent on the sample stimulus presented before (Fig. 3). Again, the discrimination could be simple or delayed and conditional. The difference, however, is the non-spatial nature of the sample stimuli. There is of course a certain spatial element in that the response at the time of comparison is spatial in nature (left or right lever), but the spatial component of the task is clearly reduced when compared with DA or D(N)MTP.

Most important, however, is the fact that the set of stimuli used during the comparison phase (two operand) differs from the set of stimuli used during the sample phase (for example, two tones). As a consequence it difficult to see how DCD tests recognition. Thus, the animal is not directly recognizing the stimulus at the time of choice and the task may be more readily classified a recall paradigm. Only if we assume prospective coding to be involved in the recognition process it would be possible to consider this task a recognition memory paradigm, but evidence for this possibility is missing. A further weakness inherent to this task is that there are two sets of information which could be remembered: the subject could remember the sample stimulus (non-spatial) or, alternatively, translate information about the sample stimulus into a response instruction (for example, high frequency press right; see Honig and Dodd, 1983; Pontecorvo et al., 1996). Thus, we face problems similar to those discussed earlier for DMTS/DNMTS—a problem which is inherent to all delayed response paradigms.

As discussed earlier, there is another problem common to all delayed response tasks in that it is possible for the subject to adopt mediating strategies. Although attempts to minimize those strategies have been reported (e.g. Herremans et al., 1994; Stanhope et al., 1995), they cannot be excluded and, therefore, present another factor which can confound the reliable assessment of recognition memory in the rat when tested in this type of paradigm.

5.2. Delayed Comparison Paradigms

Overt mediating strategies are unlikely to operate in delayed comparison tasks, since it is unknown to the animal where to respond prior to the end of the delay (the possibility that some covert mediating strategy, unknown to the experimenter, is used can of course never be excluded). Because of this, it is also rather unlikely for the subject to translate information about the sample stimulus into a response instruction prior to the delay. Thus, delayed comparison tasks should provide a way out of the dilemma and allow the unambiguous measurement of recognition memory in rats.

The “prototype” delayed comparison tasks are the delayed matching and delayed non-matching to sample procedures (DMTS/DNMTS). Those tasks have been widely used to study recognition memory in human and non-human primates as well as in pigeons. Most of these delayed matching paradigms are characterized by pairing of events, i.e. of sample and comparison stimuli. We shall therefore refer to those paired event tasks as DMTS or DNMTS. They differ from continuous matching or non-matching to sample tasks (CMTS/CNMTS) in that trial stimuli in continuous non-matching are continuously presented rather than paired.

5.2.1. Delayed Paired Matching to Sample (DMTS)/Delayed Paired Non-matching to Sample (DNMTS)

In these tasks, a sample stimulus is presented, withdrawn and, following a delay, comparison stimuli are displayed (Fig. 4). The subject has to discriminate between these comparison stimuli and to respond according to a matching or non-matching rule, respectively. Since the subject does not know the location of the correct response until the end of the delay, it must either remember a representation of the stimulus or an internal/symbolic equivalent thereof (Pontecorvo et al., 1996). This in turn makes it likely that it is recognition memory which is activated during the choice phase.

DMTS paradigms can be described as multiple choice (with rats, normally two choices are presented), discrete trial, paired event paradigms. They can only be solved by a delayed conditional discrimination at the time of choice. This discrimination can involve small sets of stimuli (delayed conditional discrimination) or large sets. In the latter condition, stimuli become trial-unique—or at least pseudo-trial-unique. Using trial-unique stimuli, the subject has to develop a relative class concept in order to respond correctly to new pairs of stimuli. Using pseudo-trial-unique stimuli, it is also possible that subjects respond according to a concept. However, rote learning is possible under these circumstances, i.e. the rat may learn which discriminations are associated with reinforcement (Thomas, 1996). Then, concurrent discrimination and/or chaining could be the mnemonic processes which are essential for correct responding.

There are several (non-operant) studies employing large sets of pseudo-trial-unique stimuli. In other words, these studies test recognition at another mnemonic level than the delayed response paradigms.
Fig. 4. Delayed paired matching to sample (DMTS). A trial in DMTS starts with a sample phase, in which only one stimulus of a set of stimuli is presented. The figure shows examples with two different stimuli, sample A and sample B. After a delay the choice phase begins with the presentation of both stimulus A and stimulus B, whereby the spatial location of the stimuli is not fixed (for example, trial 1 vs trial 2). The rat is rewarded for responding towards the stimulus which matches the sample that was presented in the sample phase in case of a DMTS task (correct response). Presentation of reinforcement is indicated by ‘+’, while absence of reinforcement is indicated by ‘–’. After an intertrial interval the sample phase of the next trial is initiated with a randomly determined next sample/position. In our example trial-unique stimuli are employed (A vs B, C vs D, E vs F, etc.; note the difference to CNMTS, Fig. 5). Alternatively, the same stimuli could be employed on every trial (small set of stimuli). This discrete protocol is maintained throughout all following trials. This figure shows four examples of correct responses (trials 1, 2, 4 and 5) and one example of an incorrect response (trial 3). In case of delayed paired non-matching to sample (DNMTS) the animal is rewarded if its response is not towards the same stimulus as in the sample phase (incorrect response), but towards the stimulus that does not match the sample presented during the sample phase (correct response). In the example shown reinforcement (‘+’ and ‘–’) would be reversed in case of DNMTS.
There is, however, no rodent D(N)MTS study reported in the literature where true trial-unique stimuli have been used. It is therefore not possible to unambiguously conclude that the use of class concepts was taxed in these tasks. However, trial-unique stimuli have been successfully employed in CNMTS (Shaw and Aggleton, 1993), and we can infer from the results obtained in this task which taxes processes comparable to those activated in DNMTS that rats are able to employ such concepts.

Unfortunately, fully automated, operant DMTS/DNMTS tasks for rodents, using delays longer than 4 sec, appear to be difficult to establish, although there is no obvious reason why rats should be unable to master such tasks with longer retention intervals. Indeed, more recent attempts point towards new directions for achieving this aim. Using recurrent complex visual stimuli (pseudo-trial-unique), Nakagawa (1993) trained rats up to delays of 2 sec on DMTS as well as DNMTS. Others also demonstrated that rats can acquire matching to sample (Iversen, 1993; Andrews and Janssen, 1996) as well as non-matching to sample (Bussey et al., 1994; Gaflan and Eacott, 1995) rules by responding to recurrent visual stimuli either projected onto small screens (Andrews and Janssen, 1996), computer-generated and presented on a video monitor (Bussey et al., 1994; Gaflan and Eacott, 1995), or by using flashing and steady lights displayed on horizontally arranged nose keys (Iversen, 1993). However, Andrews and Janssen (1996) failed to train their rats to respond above chance level with delays longer than 0 sec, and the data reported by Iversen (1993), Bussey et al. (1994) as well as by Gaflan and Eacott (1995) are devoid of any delay, thus leaving the question unanswered as to whether rats can reliably perform delayed MTS or delayed NMNTS in their paradigms. Reasons for the failure of rats to respond reliably with longer delays in the studies by Nakagawa (1993) and Andrews and Janssen (1996) could be a submaximal discriminability of stimuli, the influence of proactive or retroactive interference (see Pontecorvo et al., 1996, and below), or the discontinuity between stimulus, response and reinforcement (Ennaceur et al., 1997a).

More recently, Givens and McMahon (1997), using operant chambers equipped with three levers, reported successful DMTS training in rats performing well above chance with delays up to 4 sec, where the sample stimulus consisted of a lit or unlit centre light and the comparison stimuli were lit or unlit side lights above the levers. Moreover, Ennaceur et al. (1997a) describe an automated DNMTS paradigm for rats, using repeated, complex visual stimuli, and delays ranging from 0 to 12 sec. Their rats performed above chance at all delays. However, they did not show a consistent, delay-dependent decline. As pointed out by Iversen (1993), rats can learn to choose accurately in DMTS without being able to match the stimuli, but by learning a number of different conditional responses. Indeed, this strategy could confound performance in tasks using small sets of stimuli, not only in delayed comparison, but also in delayed response paradigms—a potential caveat in otherwise exciting results.

Using non-automated or semi-automated tasks, other authors have claimed greater success in training rats on DMTS or DNMTS. Rothblat and Hayes (1987) trained rats on DNMTS, with a large, pseudo-trial unique stimulus set (300 three-dimensional junk objects), by using a straight runway with a goal box attached at one end, being utilized for both sample and choice presentation. On each trial, a sample object was placed over a central food well which was baited with a food pellet, and the animal had to displace the sample object for reward. Then the animal was removed to the start area of the runway, two stimuli, the previously chosen sample object and a new object, were placed over two food wells in the goal box, and the animal had to displace the new object in order to obtain a food pellet. Mumby et al. (1990) described a DNMTS paradigm with delays up to 10 min, which differs from the DNMTS task developed by Rothblat and Hayes (1987) in that rats were tested using a test box with a central delay chamber and two separated ends of the box, containing a sample stimulus, which, on first glance, represents a delayed non-matching to sample strategy. However, Herremans et al. (1995) demonstrated that rats are unlikely to remember the sample object, but learn to discriminate between the comparison stimulus in Mumby’s paradigm—a discrimination likely to be based on olfactory scents, possibly introduced by handling the objects (see also Kolb et al., 1984, for similar criticism, but Mumby, 1995, for contradictory results). Other experimental factors which have been suggested to interfere with the mnemonic demands of such non-automated tasks are inadvertent auditory and visual cues during positioning of the objects (Mumby, 1995). Beck and Kalynschuk (1992) described another variant by presenting two identical sample stimuli in one compartment of the chamber and a third, matching and one non-matching stimulus during comparison in the other end of the chamber, thereby preventing scent-marking by the rat. However, care has to be taken to exclude the possibility of scent marking by the experimenter. Thus, the possibility that rats use simple odour discrimination instead of memory for large stimulus sets needs to be controlled for in these tasks. In fact, this is true for all studies where three-dimensional objects are (manually) placed into a test box and implies that great care has to be taken in order to avoid such confounding, even if the comparison stimulus which is identical with the sample is new (i.e. if new sets of identical stimuli are employed). However, the inadvertent provision of stimuli can be reduced by a range of precautions. First, a new pair of objects, one being identical with the sample object, has to be used for comparison. Second, the objects have to be inserted into the test apparatus simultaneously or at least in varying order, and, third, left/right positions of the two objects have to be randomly determined so that orientation by olfactory or other cues is unlikely to develop. These
factors have been considered in at least some studies (see for example, Steele and Rawlins, 1993, for an explicit description of how to avoid or at least minimize these potential confoundings).

More recently, a discrete-trial DMTS procedure for rats has been described, scheduled in a T-maze and using a small set of olfactory stimuli (Ravel et al., 1992). In this paradigm, a sample odour stimulus is presented in the stem of the T-maze, and, after a delay, the two comparison stimuli are displayed at the choice point.

An object recognition task which differs in design from those discussed above has been developed by Ennaceur and Delacour (1988). In this paradigm, which can be regarded as a spontaneous NMTS task (Aggleton, 1993), rats are first exposed to a sample object which they may explore for a certain time. Following a delay, they are allowed to explore two new stimuli, one object being identical with the sample, i.e. familiar, the other being new. No reinforcement is provided and the time to explore measured; animals normally spend more time with the new stimulus (Ennaceur and Delacour, 1988). A variant of this task has been described by Della et al. (1992), where novel and familiar objects were placed at the ends of arms of a Y-maze. The object recognition paradigm differs from those tasks described previously in that there is no reference memory component involved as the animals explore the objects spontaneously and no appetitive (reward) component that could also vary. Moreover, as no response rule needs to be taught, the animals are better matched—differences in the number of training-trials required to reach similar levels of accuracy may already reflect differences in cognitive function. A related paradigm is the social recognition task where animals are first exposed to a juvenile, and, following a delay, are re-exposed to the same juvenile rat (Dantzer et al., 1987). Animals tend to spend less time investigating the familiar juvenile on second exposure, which is taken as an index of olfactory recognition. A disadvantage of this paradigm, however, is its lack of choice, thus rendering it comparable to go/no-go paradigms, where it is not possible to distinguish a “true” mnemonic error from non-responding due to other reasons. Engelmann et al. (1995), in a variation of this task, introduced a second, novel juvenile, thus avoiding this potentially confounding factor. However, social recognition also includes a spatial component as juveniles will move around—a variable which can be separately manipulated in object recognition by re-exposure to the same objects but presentation of these objects in a novel place of the test chamber (Ennaceur et al., 1997b).

A range of other non-automated MTS and NMTS tasks have been reported for testing rats. Raffaele and Olton (1988) trained rats in a runway expanding into a goal area into which two interchangeable goal boxes could be placed. These boxes differed in brightness and tactile modality. In a first, forced run, the animal had to explore one of the two boxes and was returned to the start of the runway. Then the position of the boxes was changed in pseudorandom order and, under matching conditions, the rat had to enter the box visited before. Unfortunately, this design allows the animal to use olfactory stimuli. Furthermore, the paradigm lacks clearly specified delays (or, in fact, with just one delay between sample and comparison stimulus presentation, resembling the time required to place the rat from the goal to the start box). Given that mnemonic performance depends on the length of the delay, this is an obvious problem of the task, since a distinction between memory and non-specific factors is not readily permitted.

5.2.2. Delayed Continuous Non-matching to Sample (CNMTS)

Aggleton (1985, 1993) developed a CNMTS paradigm where rats are trained in a Y-maze to select a goal arm that contains an unfamiliar three-dimensional junk object over the other goal arm that contains an object similar to a sample object placed in the start arm (Fig. 5). This task differs in that stimuli are firmly affixed in hardboard boxes which are slotted into the ends of the maze arms. Since the objects need not to be touched by the experimenter it can be argued that the use of simple odour discrimination is less likely in this task. Furthermore, it has been demonstrated that rats can perform at high accuracy level in this paradigm if stimuli are purely visual (Aggleton, 1996).

The goal arm chosen by the rat becomes the start arm for the following trial, i.e. the non-matching comparison stimulus subsequently becomes a sample stimulus. In other words, this task resembles a continuous non-matching paradigm which distinguishes it from the discrete-trial tasks described above. This can be regarded as being a problem with Aggleton’s paradigm as it is difficult to schedule a matching version since then the animal would always alternate between one set of identical stimuli. However, this task is not only of value for assessing delayed non-matching performance, but has been successfully employed for studying recognition of lists of up to 32 items in rats (Steele and Rawlins, 1989, 1993), and it is possible to distinguish between recency and familiarity (Shaw and Aggleton, 1993).

5.2.3. Delayed Paired Comparison (DPC)

Wallace et al. (1980) trained rats in an operant, Konorski-type (Konorski, 1959), discrete-trial DPC task, where a sample stimulus (a tone or a light) was followed, after a delay, by a single comparison stimulus (a tone or a light). Rats where reinforced for responding when sample and comparison stimuli matched and for withholding responding when stimuli differed, i.e. to follow a go/no-go rule (Fig. 6). Unfortunately, performance dropped to chance level with delays as short as 5 sec. This, however, may be a result of non-specific factors such as training procedure or rat strain since Winocur (1992), using a brightness discrimination, was able to show that rats can maintain accuracy levels above 70% correct responding at delays up to 15 sec. Indeed, it has been shown more recently that accuracy in this task depends on the rat’s ability to determine whether they are within or between trials during an interstimulus interval, an ability that depends on the duration of the retention interval,
Fig. 5. Continuous non-matching to sample (CNMTS). A session starts with a start sample phase, in which the animal from a start situation responds towards one stimulus (for example, a junk object A1 at the end of an arm in a Y-maze). This stimulus becomes the sample for the following first trial. In the figure this "functional transformation" is indicated by the circle-ended dotted lines. After a delay the rat has to choose between two stimuli (for example, between object A2 which is a copy of object A1—i.e. identical to, but not the same as A1—placed in the arm to its left and object B1 which is placed in the arm to its right and is clearly different from objects A1 and A2). If the animal responds towards the stimulus (for example, object B1) that does not match the sample (object A1) reinforcement will be given, indicated by "+". If the animal chooses the stimulus that is identical to the sample (object A2) no reinforcement will follow, indicated by "-". This reinforcement protocol is maintained throughout all following trials. Note that in this paradigm the delay period coincides with the intertrial interval, a feature that constitutes the continuous character of this task. The figure shows some of the possible correct (trials 1, 3 and 4) and incorrect (trial 2) responses (arrows).
Fig. 6. Delayed paired comparison (DPC). This task starts with a sample phase wherein one of two stimuli, either sample A or sample B (often a tone and a light), is presented. The trial then automatically continues with a delay, after which the choice phase begins in which again one of the two stimuli, either stimulus A or stimulus B is presented. The animal then has to compare (start situation) the presented stimulus with the sample presented earlier during the sample phase: to obtain reinforcement the animal has to respond ("GO") by for example, pressing a lever when sample and stimulus match, whereas it has to withhold responding ("NO-GO") when the presented stimulus does not match the sample. Presentation of reinforcement is indicated by " + " while absence of reinforcement is indicated by " - ".

After an intertrial interval the sample phase of the next trial is initiated with a randomly determined next sample. This protocol is maintained throughout all following trials.
5.3. Delayed Continuous Comparison (DCC)

A continuous type of operant delayed comparison task has been described by Pontecorvo (Pontecorvo, 1983; see also Pontecorvo and Clissold, 1993, and Pontecorvo et al., 1996, for overviews; Fig. 7), where either a tone or a light are presented and the animal has to respond on one lever if the stimulus is the same as in the previous trial (match trial) or differs from that in the previous trial (non-match trial). Information where to respond cannot easily be recalled but the stimulus must be recognized, since it is not possible for the subject to translate information about the stimulus into a response instruction prior to the presentation of the comparison stimulus. This task can therefore be classified an operant recognition memory paradigm for rats, measuring memory for recency, and delays up to 40 sec can be scheduled without difficulties. However, a problem inherent to the maze-based procedures, namely the inability to test performance at zero-delay, remains in this operant task, since the subject must respond and be rewarded before the next trial begins.

Variants of this procedure have been reported, and of special interest is a paradigm developed by Otto and Eichenbaum (1992). These authors used olfactory instead of auditory/visual stimuli and delays up to 60 sec. They reported that their animals learned the task rapidly within just four sessions and, most importantly, stimulus set sizes up to 16 different odours could be used. However, animals had to respond according to a go/no-go rule, i.e. in Otto and Eichenbaum’s paradigm, rats had to respond if stimuli non-match and to withhold responding under matching conditions. The problems arising from this approach have been outlined above.

Thus, there are many paradigms for the measurement of recognition memory in rats and all of them have certain advantages but also drawbacks, some of them so serious that an unambiguous interpretation in terms of recognition memory is difficult. Despite numerous problems, however, we can conclude that it is possible to assess recognition mem-
Fig. 7. Delayed continuous comparison (DCC). This task starts with a *start sample phase* wherein one of two stimuli, either *sample A* or *sample B* (often a tone and a light), is presented. The animal has to respond to one of two possible operandas, usually levers, where a response to the one lever indicates that the sample and stimulus were matching ("match choice"), whereas a response to the other lever indicates that sample and stimulus did not match ("non-match choice"). Upon the end of the following delay the next trial is initiated and the animal is presented a randomly selected stimulus, either *stimulus/sample A* or *stimulus/sample B*. This stimulus either matches the sample presented during preceding trial or is different from the sample presented during the preceding trial. The animal has to decide whether or not this presented stimulus matches and subsequently has to press the corresponding lever, i.e. the match choice lever or the non-match lever, respectively. Subsequent presentation of reinforcement upon a correct choice is indicated by "+" while after an incorrect response no reinforcement is given, indicated by "−". This protocol is maintained throughout all following trials. Note that in this paradigm the delay period also separates the consecutive trials, a feature that constitutes the continuous character of this task. The figure shows an example of a possible response sequence (arrows), where the animal chooses correctly on trials 1, 2 and 4, while an incorrect response is made on trial 3.
rodents, and although it is reasonable to suppose that rats should be able to perform successfully in such paradigms, there are at present no reports providing convincing evidence in favour of this suggestion. Other operant and maze-based delayed comparison tasks have been employed more successfully, but each of them suffers one or the other drawback.

The measurement of recognition memory has been more successful in other species, including pigeon, monkey and human, and true operant D(N)MTS paradigms are readily available for these species. The use of less than ideal paradigms in rats or even the use of paradigms which may tax processes other than the rat’s recognition memory, however, is likely to contribute to difficulties in inter-species comparison and to the poor predictive validity of the results obtained from these studies.

REFERENCES


