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ORIGINAL PAPER

Behavioral evidence for two distinct memory systems in rats

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Abstract
Serial reaction time tasks, in which subjects have to match a target to a cue, are used to explore whether non-human animals have multiple memory systems. Predictable sub-sequences embedded in the sequence of cues are responded to faster, demonstrating incidental learning, often considered implicit. Here, we used the serial implicit learning task (SILT) to determine whether rats' memory shows similar effects. In SILT, subjects must nose-poke into a sequence of two lit apertures, S1 and S2. Some S1 are always followed by the same S2, creating predictable sequences (PS). Across groups, we varied the proportion of PS trials, from 10 to 80%, and show that rats with more PS experience do better on them than on unpredictable sequences, and better than rats with less experience. We then introduced test trials in which no S2 was cued. Rats with more PS experience did better on test trials. Finally, we reversed some sequences (from predictable to unpredictable and vice versa) and changed others. We find that rats with more PS experience perseverate on old (now incorrect) responses more than those with less PS experience. Overall, we find a discontinuity in performance as the proportion of PS increases, suggesting a switch in behavioral strategies or memory systems, which we confirm using a Process Dissociation Procedure analysis. Our data suggest that rats have at least two distinct memory systems, one of which appears to be analogous to human implicit memory and is differentially activated by varying the proportion of PS in our task.

Keywords Implicit memory · Explicit memory · Rat · Serial reaction time task · SILT · Process dissociation procedure (PDP)

Introduction

Recently, there has been increasing discussion of the multiple systems that subserve memory (Squire 2007). Though most of this discussion has centered on human memory, there is growing evidence for distinct memory systems in non-human animals as well (Eichenbaum et al. 1994; Tu et al. 2011; Anderson et al. 2014). One of the primary distinctions made by researchers is between declarative (or explicit) and non-declarative (or implicit, or procedural) memory (Roediger et al. 2008). By standard definitions, declarative memory is representational, and is expressed through (conscious, verbal) recollection, while non-declarative memory is expressed through performance and reflects how we physically interact with the world (Squire 2007). The distinction between the two forms of memory thus rests mostly on whether they are accessible (declarative) or not accessible (non-declarative) to conscious recall, making it extremely difficult to demonstrate their existence in non-human animals (Hampton et al. 2020).

There have nonetheless been a few attempts to identify animal correlates of the distinction between explicit and implicit memory (e.g., Basile and Hampton 2011). One method used to distinguish the two processes is the Serial Reaction Time Task (SRTT). In SRTTs, which have been used extensively in the study of both human and non-human memory (Robertson 2007), subjects are required to respond to seemingly random sequences of stimuli. Unbeknownst

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to the subjects, some or all of the sequence is fixed and therefore predictable. Both human and non-human subjects become faster at responding to these predictable subsequences (e.g., Nissen and Bullemer 1987; Turner et al. 2005; Heimbauer et al. 2012), even when—in humans—they verbally report no knowledge of fixed sequences in the stimulus chain (Nissen and Bullemer 1987). When switched to truly random sequences, reaction times increase (sometimes referred to as an interference effect; e.g., Christie and Hersch 2004). These results strongly suggest that subjects implicitly encode the fixed sequences, and can predict an upcoming stimulus, reducing their reaction time.

A key aspect of SRTTs is that subjects are not required to learn or remember the fixed sequences (Turner et al. 2005): all responses are individually cued, and subjects can solve the task without predicting upcoming stimuli. Learning the sequences in this task, and sometimes all implicit learning, is, therefore, considered incidental (Seger 1994; Drucker et al. 2016). Several studies have demonstrated that non-human animals nonetheless learn the sequences in such tasks (Turner et al. 2005; Locurto et al. 2010, 2013), and that the sequences are learned at a motoric level (i.e., abstract stimulus features of the sequence do not seem to drive behavior; Procyk et al. 2000; Turner et al. 2005; Reber 2013; Drucker et al. 2016).

It has been suggested that procedural memory performance is mediated by the striatum, while declarative memory resides in the temporal lobes and diencephalon (e.g., Squire 2007). However, lesions of the striatum in rats do not cause a specific impairment to implicit learning (though they do cause a general motor impairment; Jay and Dunnett 2007). Similar non-specific deficits on sequence learning result from reduction of dopamine levels in the striatum (Eckart et al. 2010), lesions of the premotor and supplementary motor areas (Brooks and Dunnett 2009), lesions of the hippocampus or caudate (Christie and Dalrymple-Alford 2004), and—in pigeons—deactivation of the nidopallium (Helduser and Güntürkün 2012). As far as we are aware, no-one has successfully induced specific deficits in implicit learning by lesioning any region of a non-human brain. Lesions of the perirhinal cortex in monkeys do appear to cause deficits in trial-specific (possibly explicit) memory while sparing habitual responses, which may be implicit (Tu et al. 2011).

The Serial Implicit Learning Task (SILT; Jay and Dunnett 2007) is a simplified SRTT which has primarily been used with rats and mice (Jay and Dunnett 2007; Brooks et al. 2007, 2012; Brooks and Dunnett 2009; Trueman et al. 2005, 2007, 2008). The task consists of requiring subjects to respond, by nose-poking into illuminated apertures, to pairs of stimuli presented sequentially. On some trials, the sequence of apertures is random; on others, it is predictable (Jay and Dunnett 2007). The experiment is usually carried out in a 5-aperture chamber, in which the apertures are labeled A–E. On some trials, the first aperture illuminated (e.g., A) could be followed by any of the remaining four apertures; on other trials, the first stimulus (e.g., B) will always be followed by a specific second stimulus (e.g., D). In many implementations of this task, including ours, two of the initial stimuli (B and E) have predictable consequents (D and C, respectively), while the remaining three initial stimuli (A, C, and D) can be followed by any one of the four remaining apertures. The selection of the first stimulus is often uniform, so that predictable sequences make up 40% of all trials (Jay and Dunnett 2007).

The predictability of some sequences in the SILT is assumed to be learned implicitly, by analogy with human SRTT data. If this is true, it suggests that subjects should, under the right circumstances, be capable of other forms of learning, which we might label explicit, again by analogy with human memory. As noted above, the problem with attempting to identify this distinction in non-human animals is that our only access to their internal states is via their interactions with the world, i.e., their behaviors. Non-verbal animals, including young human children, do not report on their conscious recollections (but see Anderson et al. 2014; Hampton et al. 2020). We avoid the charged issue of whether or not non-human animals are conscious and merely note that it is at least conceivable that they might have multiple memory systems without being conscious.

Here, we attempted to use the SILT to explore whether rats can be said to have two different memory systems, and in what ways these memory systems appear to be functionally homologous to the declarative and non-declarative systems of human memory. We did this by varying parameters of our task, such that it engaged the different memory systems to varying degrees. First, we replicated Jay and Dunnett's (2007) experiment but varied the proportion of trials that were predictable. We assumed that if rats have two memory systems, one of which is engaged only by frequently recurring patterns, then changing the proportion of predictable trials might identify the threshold beyond which this system is active. Next, we introduced uncued test trials, on which the first aperture to be responded to (S1) was one of those with predictable consequents during training, but a second lit aperture (S2) was not provided. We assumed that rats with explicit memories of the predictable sequence, or who had more experience of predictable trials, would be better at generating the second half of the sequence without cueing, similar to recall or priming tests in humans. Finally, we reversed some of the contingencies, making one formerly predictable S1 unpredictable, one formerly unpredictable S1 predictable, and changing the sequence of the other predictable S1. We predicted that changing an acquired response would be easier if the behavior were driven by explicit processes than if it was under the control of a procedural

mechanism. We then subjected all our results to an analysis designed to tease apart the influence of each memory system on behavior.

Jacoby (1991) has argued that the interpretation of tests of implicit and explicit memory is complicated by the fact that our behavioral tests are unlikely to be "process pure". In other words, most tests of memory likely engage both memory systems to some degree, so that accuracy on these tests cannot be taken as an unbiased measure of the functioning of a specific memory system. Jacoby (1991) has suggested a Process Dissociation Procedure (PDP) to overcome this difficulty, which has recently been used to explore multiple memory systems operating in non-human animals (Hampton et al. 2020). Under the assumption that both processes contribute to most memory tests, the procedure identifies one test in which greater involvement of both processes should improve results (a facilitation test), and one test in which the two processes should motivate opposing responses (a conflict test). For example, our uncued test trials could be considered facilitation tests, since we should expect improvements in rats' ability to generate the correct response with increasing explicit *or* implicit memory for the sequence. On the other hand, our reversal trials are an example of a conflict test, since explicitly encoding the new rule should lead to improved performance, whereas procedural memory for the old sequence (which is presumably harder or slower to reverse) should lead to errors. PDP allows performance on both types of tests to be combined to estimate the contribution of each process to performance on the task (see "Methods"). We applied this procedure to our data in an attempt to estimate whether changing the proportion of predictable sequence trials engaged different memory systems across our groups.

Methods

Subjects

Subjects were 45 male Sprague–Dawley rats [Crl:CD (SD); Charles River Laboratories Inc., St. Constant, QC, Canada], approximately 60 days old at the start of the experiment. A further 4 rats failed to acquire the task and were excluded from all analyses. Subjects were pair-housed upon arrival in the lab and, a week later, were transferred to individual cages. Rats were handled for at least 10 days prior to the start of the experiment. The colony room was maintained at 21–22 °C on a 12-h reversed light–dark cycle (lights off at 7:00 a.m.). During the experiment, animals were fed a restricted diet to maintain their body weights at 90% of their free-feeding levels, and given water ad libitum. The procedures used followed the Canadian Council on Animal Care guidelines and were approved by the Wilfrid Laurier University Animal Care Committee (AUP R16006).

Apparatus

Animals were trained and tested in modular operant chambers (ENV-008, Med Associates, St. Albans, VT). Each chamber was constructed of aluminium and was placed inside a fan-ventilated sound-attenuating cubicle. Chambers were 29.5 × 25 × 18.7 cm, and had a stainless steel-rod floor. The back wall of each chamber was curved and contained five 2 × 2 cm apertures (5 unit curved nose poke wall, ENV-115A-07, Med Associates, St. Albans, VT), spaced equidistant from each other and the side walls, 2 cm above the chamber floor. We refer to these apertures by the letters A to E. Each aperture was equipped with an LED and a photocell sensor to detect nose-pokes. The opposite wall of the chamber contained a food magazine through which 45 mg grain-based food pellets (FO165, Bio-Serv) could be delivered, and which also contained an LED. A house-light was mounted above the food magazine.

Procedure

Our experimental procedures closely followed the SILT as described by Jay and Dunnett (2007). The task requires subjects to nose-poke into two apertures, denoted S1 and S2, that are illuminated in order, following which they receive a food pellet reward. When S1 was aperture A, C, or D, the choice of S2 was selected at random with an equal probability of occurring at any of the remaining 4 locations. We refer to these as unpredictable sequence (US) trials. When S1 was aperture B, S2 was always aperture D, and when S1 was E, S2 was always aperture C. We refer to these as predictable sequence (PS) trials. The two predictable sequences were both "2-hops" away, i.e., in both cases S2 was two apertures away from S1.

Rats were divided into five groups that varied in the proportion of their PS trials. We label groups by this percentage: 10% ($n = 9$ rats), 20% ($n = 8$), 40% ($n = 10$), 60% ($n = 8$), and 80% ($n = 10$). Group 40% constitutes a direct replication of Jay and Dunnett's (2007) experiment. Rats completed one session per day, 7 days a week, for the duration of the experiment. All sessions were conducted between 9:00 a.m. and 2:00 p.m. (i.e., during the rats' dark phase). Rats progressed through the following seven phases of the experiment. In all phases, rats were first placed into the chamber with the house light on. The start of the session was signalled by a 5 s interval with all the lights off. Phases 1–4 were identical for all groups.

1. Pre-exposure: subjects were given a small dish in their home cages containing approximately 100 pellets each day until they consumed all the pellets for 2 days in a row. All rats completed this phase in 2 days.
2. Habituation: subjects were placed into the testing chamber. 2 pellets were placed into each of the five nose-poke apertures and 10 pellets were placed into the food cup. Sessions lasted for 20 min, during which all the aperture lights were illuminated. This was repeated until the rats consumed all the pellets. Rats spent 1–2 days (mean 1.2 ± 0.41 SD) in this phase.
3. Response shaping: on the first day of this phase, subjects received non-contingent pellets at the food magazine every minute, as well as any time they nose-poked into any aperture. Sessions lasted for 30 min or until rats had consumed 50 pellets, whichever occurred first. On the following day, the same procedure was followed but without the non-contingent pellets. Rats continued on these two session types until they were reliably consuming 50 pellets in under 30 min. At this point, the criterion was increased to a maximum of 40 min or 100 pellets. Rats spent 3–7 days (mean 4.35 ± 0.88 SD) in this phase.
4. Nose-poke training: subjects received a pellet for nose-poking into one randomly selected aperture that was illuminated. Nose-pokes into non-illuminated apertures were not rewarded and were ignored. Sessions lasted for 40 min or until rats collected 100 pellets, whichever occurred first. Subjects continued this phase until they collected 100 pellets in under 40 min. Following this, the procedure was altered so that nose-pokes into non-illuminated apertures resulted in a 5 s time-out (all lights off, no rewards available). Rats continued this phase until they collected 100 pellets in under 40 min. Rats spent 1–2 days (mean 1.7 ± 0.47 SD) in this phase.
5. SILT: in this phase, the start of each trial was signalled by switching on the stimulus light S1 in one of the apertures (A to E), selected in a pseudo-random sequence (see below). S1 remained illuminated until a nose poke was detected in the lit aperture, whereupon the S1 stimulus was switched off and a different aperture, S2, was illuminated. Following a correct response to the S2 aperture, the stimulus light was turned off, a food pellet reward was delivered to the magazine and the magazine light illuminated. The correct trial was terminated upon detection of a nose-poke into the magazine, and the magazine light was turned off. Responses to an incorrect aperture or to the magazine during S1 or S2 resulted in errors that were signalled by a 5 s time out, during which the house light was illuminated and all other lights turned off. Following reward collection on correct trials or time out on error trials, there was a 5 s inter-trial interval in the dark, with all lights (including the house light) switched off, prior to the start of the next trial, signalled by the illumination of the next S1.

The proportion of PS trials was varied across groups. The selection of S2 was pseudorandom on US trials (when S1 was A, C, or D) and was determined by S1 on PS trials (B→D and E→C). Possible trial types were pseudo-randomized in blocks of 20 trials.

a. For group 10%, in each 20-trial block: 1 trial had S1 = B; 1 trial had S1 = E; and 18 trials had S1 = A, C, or D (6 trials each).
b. For group 20%, in each 20-trial block: 2 trials had S1 = B; 2 trials had S1 = E; and 16 trials had S1 = A, C, or D (5 or 6 trials each, counterbalanced).
c. For group 40%, in each 20-trial block: there were 4 trials of each possible S1.
d. For group 60%, in each 20-trial block: 6 trials had S1 = B; 6 trials had S1 = E; and 8 trials had S1 = A, C, or D (2 or 3 trials each, counterbalanced).
e. For group 80%, in each 20-trial block: 8 trials had S1 = B; 8 trials had S1 = E; and 4 trials had S1 = A, C, or D (1 or 2 trials each, counterbalanced).

Rats continued in this phase of the experiment until they reached a performance criterion of a 3 day average with over 80% correct on both S1 and S2, after a minimum of 10 days. Rats spent 10–26 days (mean 13.75 ± 4.83 SD) in this phase.

6. Testing: testing sessions followed the same procedure as the SILT sessions, but one trial in each 20-trial block—selected from the PS trials (i.e., S1 = B or E)—was an uncued test trial. On test trials, following a correct nose-poke to S1, no second aperture was illuminated. Rats were given 10 s to respond to any S2, after which the trial timed out and no reward was provided. Rats remained in this phase of the experiment for 10 days.
7. Reversal: Following the testing phase, rats received 10 additional days of sessions that followed the same procedure as the SILT sessions, but with altered S1 → S2 sequences. During this phase, if S1 was B, C, or D, S2 was unpredictable (i.e., selected with equal probability from among the remaining 4 choices); if S1 was A, S2 was always C; and if S1 was E, S2 was always B. In other words, two sequences remained unchanged (S1 = C or D, S2 = anything), one sequence went from being predictable to unpredictable (B → D became B → anything), one sequence altered in the opposite direction (A → anything became A → C), and one predictable sequence changed from a 2-hop to a 3-hop sequence (E → C became E → B). The proportions of trials with predictable sequences remained unchanged for each

group. Rats remained in this phase of the experiment for 10 days.

Analysis

Data collected by the MedPC software that ran the operant chambers were read into Microsoft Excel. Analyses were conducted in Mathematica (v.10.0, Wolfram Research) and JASP (JASP Team 2020).

In the reversal stage, we did not alter the proportion of PS trials, to prevent the rats suffering from generalization decrement. Thus, rats in some groups received many more PS trials in the reversal stage, and had differential experience of the various possible S1 apertures. Therefore, when comparing responses in this stage of the experiment, we present analyses of the first 50 trials for each S1, for every group. For some groups, this represents up to 10 sessions' worth; for others, only one or two.

To perform the Process Dissociation Procedure (PDP), we used data from our uncued test trials and from the reversal trials on which a predictable sequence changed from a 2-hop to a 3-hop (E → C became E → B). We assume that better implicit *or* explicit memory will facilitate performance on the uncued test trials, and that stronger implicit but not explicit memory will hinder learning the new sequence on reversal trials. In PDP terms (Jacoby 1991), the test trials constitute a facilitation test and the reversal trials a conflict test.

We first calculated the overall proportion correct on uncued test trials for each rat, and denote that value C (for Correct). We also calculated the increase in error rate after reversal, as the proportion of errors on the first 50 E → B trials (after the reversal) minus the proportion of errors on E → C trials (before the reversal, during the last 5 days of the SILT phase); we denote this value E (for Error). Then, following Jacoby (1991), we calculate for each rat a Recollection score, R = C–E, and a Familiarity score, F = E/(1–R). The Recollection score is assumed to reflect the contribution to task performance of explicit processes, and the Familiarity score the contribution of implicit memory.

We used Bayesian statistics for all tests. To compare groups and performance on different apertures or trial types, we used either one-way or repeated measures (mixed) Bayesian ANOVAs (see Wagenmakers et al. 2018), with group as the between-subjects factor. Depending on the test, the within-subjects factor was S1 aperture, S2 aperture, the distance between the S1 and S2 apertures (hop-size), or trial type (PS or US). For each analysis, we report the Bayes Factor (BF) for each model compared to the null model (i.e., we report BF_{10}). The BF is a likelihood ratio comparing two models. Thus, a BF of 5 for a model means that the data are 5 times more likely under this model than under the null; a BF of 0.1 suggests that the data are 10 times more likely under the null model. The BF thus also functions as an estimate of effect size (Wagenmakers et al. 2018). We report models with interaction terms only when the model with all main effects is better than the null model (BF > 1). We also report inclusion BFs across matched models, which measure the evidence in the data for including each predictor (or interaction term), averaged across all models (van den Bergh et al. 2020). Where main effects were substantial, we conducted post-hoc pairwise tests, which were corrected to control for multiple comparisons (using the method in Westfall 1997); we report the posterior odds for each pairwise comparison (posterior odds are also ratios, like the BF). Simple main effects were tested using individual Bayesian (one-way or paired-sample) *t* tests. To estimate the effects of explicit and implicit processes in the PDP, we used a Bayesian linear regression and we report the BF and the value of the regression coefficient with a 95% credible interval (the Bayesian equivalent of a confidence interval). We qualify all results using the adjectives suggested by Jeffreys (1961): effects with BF smaller than 3 are considered "anecdotal" evidence in favor of the hypothesis (or, if the BF is between 1 and 1/3, anecdotal evidence for the null); effect sizes between 3 and 10 (or between 1/3 and 1/10) are labelled "moderate"; between 10 and 30, "strong"; between 30 and 100, "very strong"; and BF > 100 are denoted "extreme" evidence for the hypothesis. Raw data and annotated JASP files containing all the analysis results are available in our OSF repository (https://osf.io/7hpxe/).

Results

We focused on rats' performance after they had acquired the task, during the last 5 days of the SILT phase of the experiment, and during the testing and reversal phases. For most analyses, we limited ourselves to comparing PS trials, in which S2 was always 2 apertures away from S1 (a two-hop), with US trials in which the required S2 response was also two apertures away from S1 (e.g., A → C). In other words, we removed the confounding effect of the physical distance between S1 and S2 (see also Jay and Dunnett 2007).

SILT sessions

We found strong evidence that there was no difference between groups in their accuracy on S1, but moderate evidence that rats in all groups performed better on central apertures than peripheral ones, as also found by Jay and Dunnett (2007; Figure S1A; aperture only model BF = 4.57; group only model BF = 0.09; group + aperture model BF = 0.46; inclusion BFs: group = 0.10, aperture = 4.61, group*aperture = 0.01; post-hoc tests showed odds > 10 for aperture C vs. apertures A and E only). We found

moderate evidence that rats in group 40% were faster in responding to S1 (Figure S1B; group only model BF = 6.50; aperture only model BF = 0.06; group + aperture model BF = 0.41; inclusion BFs: group = 6.53, aperture = 0.06, group*aperture = 0.01; post hoc tests showed odds > 8 for group 40% vs. all other groups; all other odds < 1). We found strong evidence that there were no differences between groups in their accuracy on S2, but very strong evidence that rats were less accurate when S2 was aperture A (Figure S2A; group only model BF = 0.05; aperture only model BF = 1.4×10^{14}; group + aperture BF = 1.4×10^{13}; group + aperture + group*aperture BF = 1.1×10^{19}; inclusion BFs: group = 0.1, aperture = 1.5×10^{14}, group*aperture = 8.0×10^{5}; post-hoc tests showed all odds < 0.2 for comparisons between groups, odds > 79,000 for aperture A vs. all others, odds > 6 for aperture E vs. apertures B, C, and D; all other odds < 0.08). We found no evidence for differences in response time across groups or between predictable and comparable (2-hop) unpredictable trials (group only model BF = 0.14; Predictable–Unpredictable [P–U] only model BF = 0.43; group + P–U model BF = 0.06).

On unpredictable sequence (US) trials, we found extreme evidence that rats were more accurate on S2 when it was closer to S1, and moderate evidence that this did not differ between groups (Figure S2B; Group only model BF = 0.03; hop-size only model BF = 2.9×10^{12}; group + hop-size BF = 1.0×10^{11}; group + hop-size + group*hop-size BF = 5.0×10^{9}; inclusion BFs: group = 0.04, hop-size = 2.9×10^{12}, group*hop-size = 0.05; post-hoc tests showed all odds < 0.11 for comparisons between groups, odds > 12 for all comparisons between hop-sizes except 3 vs. 4 [odds = 0.09]). We found anecdotal evidence that hop-size had no effect on S2 latency (Figure S2C; group only model BF = 0.77; hop-size only model BF = 0.68; group + hop-size BF = 0.83; inclusion BFs: group = 0.95, hop-size = 0.85).

We found extreme evidence that rats in all groups were more accurate on PS trials than on comparable (2-hop) US trials (Fig. 1; group only model BF = 0.14; trial-type only model BF = 40,880; group + trial-type BF = 8,011; group + trial-type + group*trial-type BF = 51,580; inclusion BFs: group = 0.20, trial-type = 42,859, group*trial-type = 6.44; post-hoc tests showed all odds < 0.22 for comparisons between groups, and odds = 15,443 for the comparison between predictable and unpredictable trial-types). There was a strong to very strong effect of trial type in groups 40% (BF = 11.60) and 80% (BF = 36.07), and a moderate effect in group 60% (BF = 3.10), but anecdotal evidence of no effect in the other groups (10% BF = 0.54; 20% BF = 0.46). As Fig. 1 shows, we found moderate evidence that groups with more experience of PS trials performed better on those trials (BF = 4.37; full post-hoc test results are given in Table S1), and moderate evidence that performance

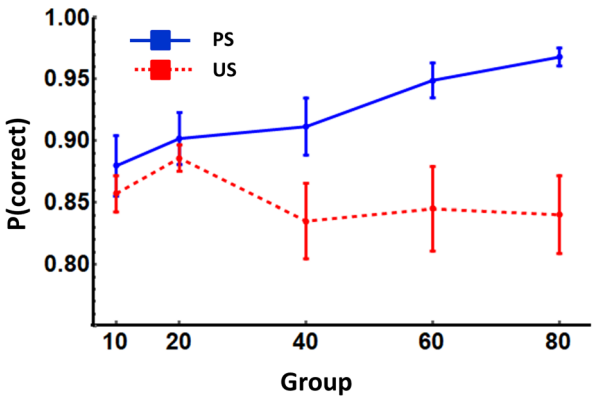

Fig. 1 SILT phase data. Proportion of correct responses to S2, by group, on predictable sequence (PS; solid blue line) and comparable (2-hop) unpredictable sequence (US; dashed red line) trials. Error bars show ± SEM (colour figure online)

on US trials did not vary across groups (BF = 0.17). We note that these data display a jump in differential performance between PS and US trials between the 20% and 40% groups. In other words, rats' improved performance on PS (compared to US) trials does not increase gradually as the proportion of those trials in the session increases. Instead, we find no effect in groups 10% and 20% (i.e., these rats are not better at PS trials than US trials), and at least a moderate effect in all the other groups. Similarly, we find no differences between groups 10%, 20% and 40% (Table S1), a weak difference between groups 10–20% and 60%, and a very strong difference between groups 10–20% and 80%. These results strongly suggest a transition between behavioral strategies or cognitive processes somewhere between groups 20% and 40%.

Test trials

We next interspersed a small number of uncued probe trials using PS S1 apertures (B or E), on which the rats were required to generate their second response in the absence of a lit S2. All groups performed poorly on these tests (Fig. 2), with the majority of errors being to the aperture immediately beside S1, in the direction of the required S2 response (i.e., rats made a 1-hop response in the correct direction, rather than the required 2-hop response). We found extreme evidence both that rats performed better when S1 was B than E, and that groups with experience of PS trials performed better on test trials (group only model BF = 2,435; S1 only model BF = 245; group + S1 BF = 8.5×10^{5}; group + S1 + group*S1 BF = 1.0×10^{5}; inclusion BFs: group = 3,468, S1 = 349, group*S1 = 0.12; post-hoc tests showed odds = 279.3 for the comparison between the two S1s, odds > 12 for groups 10% and 20% vs. groups 60% and 80%, odds = 4.9 for group 20 vs. 40%, and odds > 1.4 for group 40% vs. groups 10% and

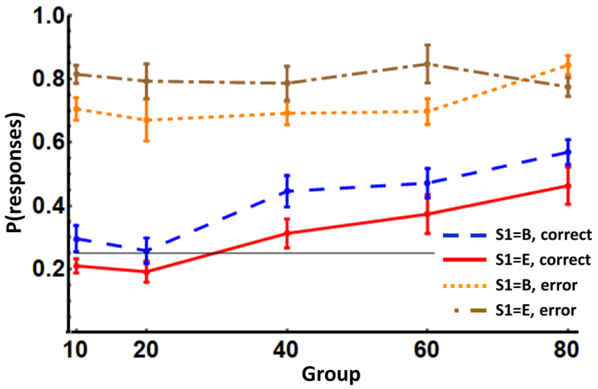

Fig. 2 Test trial results. Proportion of correct responses on uncued test trials when the S1 was aperture B (dashed blue line) or E (solid red line), and proportion of errors that were to the aperture immediately beside S1, in the correct direction (i.e., towards S2), when S1 was B (dotted orange line) or E (dash–dotted brown line). The thin black horizontal line shows chance levels. Error bars show ± SEM (colour figure online)

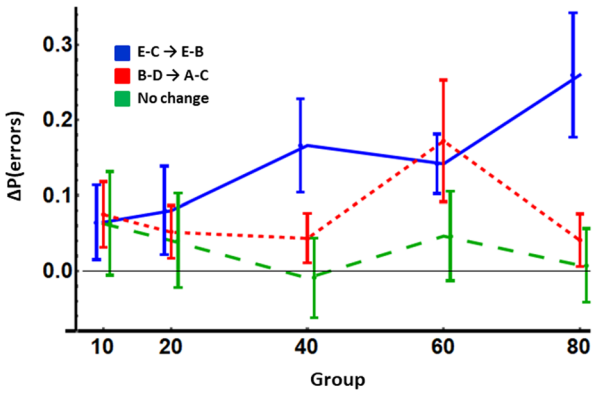

Fig. 3 Reversal trial data. The figure shows the change in error rate (proportion of trials on which rats selected the wrong S2) between the last 5 days of SILT training and the first 50 trials of each S1 in reversal. Values above 0 (thin horizontal line) indicate an increase in errors during reversal. Three types of trials are shown: a 2-hop predictable sequence that changed into a 3-hop predictable sequence (E → C/E → B; solid blue line), a predictable sequence that changed S1 (B → D/A → C; dotted red line), and the average of two 2-hop unpredictable sequences that did not change (C → E and D → B; dashed green line). Error bars show ± SEM and have been shifted along the *x*-axis for clarity (colour figure online)

80%; all other odds < 0.4). Yet again, these results appear to show a discontinuity in performance occurring somewhere between groups 20% and 40% (e.g., the posterior odds differentiating groups 10–20% from groups 60–80% are almost an order of magnitude larger than those between all other paired comparisons).

Reversal sessions

Finally, we reversed some of the contingencies, so that one formerly predictable S1 was now unpredictable, one formerly unpredictable S1 now always had a 2-hop predictable S2, and one formerly predictable 2-hop S1 now required a (predictable) 3-hop response (see "Methods").

Rats in all groups made slightly more errors on newly predictable sequences (A → C) than they did at the end of training on the original predictable sequences (B → D; Fig. 3, red dotted line), though we found only anecdotal evidence in favor of this apparent increase, and only in group 60% (overall BF = 0.37; *t* tests comparing each group's increase in errors to 0 showed BF = 1.11 for group 60%, all other BF < 1). To test for a nonspecific effect of changing the contingencies (a generalization decrement), we also compared accuracy on two sequences using S1s that did not change across phases of the experiment. When S1 was C or D, S2 could be any other aperture in both the original training conditions and under reversal (i.e., these S1s were not reversed). We compared error rates for 2-hop sequences involving these S1s (C → E and D → B) and found moderate evidence that there was no increase in errors (Fig. 3, green dashed line; C → E, BF = 0.11; D → B, BF = 0.56).

Finally, we compared the change in error rates on a predictable sequence that was changed from a 2-hop (E → C) to a 3-hop (E → B). We found moderate evidence that rats in groups with more experience of predictable trials (groups 40–80%) made more errors on the new sequence than they had on the old sequence (Fig. 3, solid blue line; overall BF = 0.56; one-way *t* test BFs: group 10%, 0.62; 20%, 0.68; 40%, 3.04; 60%, 7.19; 80%, 5.47). We note, again, the relatively abrupt change in behavior between 20 and 40% predictable trials. Interestingly, the majority of errors made on the altered sequence consisted of perseveration on the old sequence. Adding the proportion of old responses during reversal (E → C; Fig. 4, solid red line) to the proportion of correct responses (E → B; Fig. 4, dotted blue line) gives a response rate (Fig. 4, dot–dashed brown line) that almost exactly matches the proportion of correct responses on the original sequence before reversal (Fig. 4, dashed green line), and changes in the same way across conditions (Pearson-correlation $r = 0.62$, BF = 3,848).

Process dissociation procedure

To compare the effects on task performance of explicit and implicit processes, we conducted a process dissociation procedure (PDP) analysis (Fig. 5). We ran a linear regression to find the estimated change in the contribution of each process across groups. We note that it is difficult to interpret the significance of the regression coefficient, as the units in which both the original estimate and the coefficient are expressed

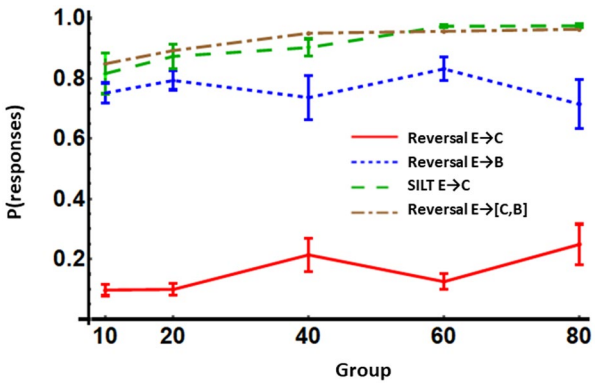

Fig. 4 Reversal errors on an altered sequence. In the reversal phase, one previously 2-hop predictable sequence (E→C) became a predictable 3-hop sequence (E→B). The figure shows the proportion of correct responses before reversal (green dashed line), the proportion of correct responses after reversal (solid red line), the proportion of perseveration errors (E→C after reversal; blue dotted line), and the sum of the correct and perseverative responses during reversal (brown dot–dashed line), which closely match the proportion of correct responses before reversal. Error bars show ± SEM (colour figure online)

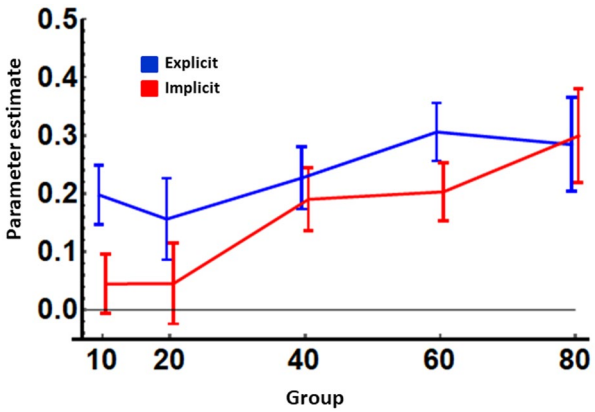

Fig. 5 Process dissociation procedure results. The figure shows the relative contributions of declarative (explicit; blue line) and non-declarative (implicit; red line) memory processes to the results of our experiment, as estimated by the Process Dissociation Procedure. Error bars show ± SEM (between individuals in each condition) and have been shifted along the *x*-axis for clarity (colour figure online)

are arbitrary (we do not report model intercepts for similar reasons). We found moderate evidence that increasing experience of predictable trials increases the use of implicit memory processes in rats (BF = 7.21, mean regression coefficient = 0.003, 95% credible interval = [0.0008, 0.006]), whereas explicit processes contribute fairly equally in all groups (BF = 0.90, mean coefficient = 0.0006, 95% credible interval = [0, 0.003]). As Fig. 5 shows, the effects of implicit processes are particularly large in groups where 40% or more of the trials are predictable sequences, which is unsurprising, given that these values are calculated from the uncued tests and reversal data, which show the same pattern. We conducted one-sample one-tailed *t* tests to estimate whether the effect of each process on performance in each group was greater than zero (Table S2). We found moderate to very strong effects of explicit processes in all groups, but no consistent change in the magnitude of this effect across groups; implicit processes, however, appear to have no effect in groups 10% and 20%, but make an increasingly important contribution in groups with a higher proportion of predictable sequence trials (see Table S2).

Discussion

To explore whether the results of serial reaction time tasks might reflect the activity of multiple memory systems in rats, we replicated the original SILT study of Jay and Dunnett (2007) while varying some parameters. We first varied the proportion of trials that were predictable, from 10 to 80%. We found that rats were better at predictable-sequence trials than unpredictable-sequence trials, and that the difference in their accuracy across the two trial types depended on the proportion of predictable trials they had during training. Importantly, the improvement in accuracy did not increase linearly with experience of predictable trials, but jumped up between our 20% and 40% groups (Fig. 1). These data suggest that rats use two different memory systems or behavioral strategies depending on how common predictable trials are, and that they switch between these strategies at about 30% predictable trials. Though we believe that in humans it is rare sequences that are learned implicitly, we have no evidence so far as to which system operates in each range in rats.

In studies of implicit and explicit memory in humans, researchers often use cued and uncued tests to explore the effects of the two systems (Roediger et al. 2008). For example, if subjects are asked to memorize a list of words, an explicit test of memory (often called a recollection test) might ask if a specific item was present in the list (i.e., a cued recall test). Alternatively, subjects could be asked to complete a missing word in a sentence with only the first letter given. This is often called a priming test and is assumed to test implicit learning (i.e., subjects are not consciously aware of being influenced by items in the list, but are more likely to use items from the list to complete the word than a subject that did not see the list). In an attempt to replicate this sort of test, we introduced uncued test trials into our experiment. We found that rats with more experience of predictable trials performed better on these test trials (Fig. 2), suggesting—by analogy with similar tests on humans—that they were more likely than the other groups to be using an implicit strategy. Again, the largest difference occurred

between groups 10–20% and groups 60–80%, suggesting that different memory systems are primarily active on either side of this divide. The evidence here, if the analogy with human memory is sound, suggests that implicit processes are engaged more strongly after more experience of predictable trials, and explicit processes after less experience.

Implicit memory, or more specifically procedural memory, underlies fixed, usually fast, responses in humans. We reasoned that a sequence of actions that was performed implicitly would be more resistant to change than one that relied on an explicit rule. We therefore reversed some of the predictable sequences in our experiment. Other than a slight general increase in errors, possibly attributable to a generalization decrement (Young and Pearce 1984), we found that rats made no more errors on a newly predictable sequence than they had at the end of training. Thus, rats are able to learn a new predictable sequence quickly (in less than 50 trials). However, rats with more experience of predictable trials made more errors on a predictable sequence that changed from 2 to 3 hops (Fig. 3, solid blue line), suggesting that they had more trouble changing or inhibiting their existing response. This explanation is further supported by the finding that the majority of errors on the new sequence consisted of perseverative responses of the old sequence (Fig. 4). This suggests that groups with more experience of predictable trials were more likely to be using an implicit strategy, which is presumably harder or slower to alter. Yet again, we find substantial evidence for the increase in error rates only in groups with 40% or more predictable trials during training, suggesting an abrupt shift in the cognitive processes involved at about 30% predictable trials.

Both our uncued test trials and reversal results point to the conclusion that implicit processes are engaged as a result of more, not less, experience with predictable sequences. This appears to be opposite to findings in humans. It is possible that this indicates that the SILT is not a good homologue for human implicit learning tasks. Alternatively, the large amounts of training and simple task that the rats underwent may have affected which memory processes were engaged in each group. In either case, our results do suggest that the existing literature on the SILT, in which—almost invariably—40% of trials have predictable sequences, should indeed engage the implicit system.

To further explore the contributions of each memory system to performance on our task, we conducted a PDP analysis (Jacoby 1991). We note that the PDP does not test whether or not there is good evidence for two separate memory processes involved in a task. Rather, the procedure *assumes* that the data result from the operation of two separate processes, and estimates the contribution of each one to task performance. In line with our previous results, the results of the analysis suggested that explicit processes are engaged more-or-less equally across all our groups, but that implicit processes make a significant contribution to performance only in groups with 40% or more predictable trials during training, and more so as the percentage increases (Fig. 5). This suggests, as noted above, that previous uses of the SILT paradigm were indeed engaging implicit memory (though not exclusively), as intended.

In conclusion, our data suggest that rats have two different memory systems—or behavioral strategies that behave like memory systems—that can be differentially activated by altering the proportion of predictable sequences in a serial reaction time task. Similar results have been obtained in other species (e.g., Basile and Hampton 2011), but not, as far as we are aware, in rats. Interestingly, we find that conditions with more common predictable sequence trials activate the system analogous to implicit memory more, rather than less. It is possible that the nature of our paradigm, which involves a simple task that is repeated many hundreds of times, is more amenable to the use of automated implicit processes to solve predictable sequence trials than comparable tasks commonly used with human subjects. Our analysis also suggests that as we increase the proportion of trials that have predictable sequences, implicit processes are engaged more strongly (or gain more control over behavioral choices), while explicit processes continue to contribute at about the same level. Since we cannot obtain verbal responses from our rats, we cannot properly label either process 'declarative' or 'explicit', but our results may constitute further evidence that non-human animals have several interacting memory systems that are engaged to different degrees depending on the task at hand.

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Declarations

Conflict of interest The authors declare that they have no competing interests.

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