Brief Communication

Double dissociation of pharmacologically induced deficits in visual recognition and visual discrimination learning

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Monkeys trained in either one-trial recognition at 8- to 10-min delays or multi-trial discrimination habits with 24-h intertrial intervals received systemic cholinergic and dopaminergic antagonists, scopolamine and haloperidol, respectively, in separate sessions. Recognition memory was impaired markedly by scopolamine but not at all by haloperidol, whereas habit formation was impaired markedly by haloperidol but only minimally by scopolamine. These differential drug effects point to differences in synaptic modification induced by the two neuromodulators that parallel the contrasting properties of the two types of learning, namely, fast acquisition but weak retention of memories versus slow acquisition but durable retention of habits.

Visual recognition, a type of cognitive memory formed by observation alone, depends critically on cholinergic muscarinic modulation of the connections linking the ventral visual stream with the rhinal cortices (Mishkin and Phillips 1990; Meunier et al. 1993; Tang et al. 1997; Turchi et al. 2005). By contrast, visual discrimination learning with long, namely 24-h, intertrial intervals (ITIs), a type of habit formation formed by trial-and-error or reward-prediction training, is mediated by a circuit connecting the ventral visual stream with the ventrocaudal neostriatum (Mishkin et al. 1984; Teng et al. 2000; Fernandez-Ruiz et al. 2001). This particular type of learning is therefore likely to depend at least in part on the dopaminergic system, which has long been known to play an important role in various forms of stimulus-response association (e.g., Fibiger et al. 1975; Robbins et al. 1990). At the same time, because cholinergic cells are embedded in the neostriatum (Mesulam et al. 1984), and because dopamine receptors were shown recently to be functionally important in rhinal cortex (Liu et al. 2004), it is unclear just how selectively the cholinergic system serves recognition memory as compared with discrimination habit formation, and, conversely, how selectively the dopaminergic system might serve habit formation as compared with recognition memory. The answer is important for determining the degree to which these two neuromodulatory systems help define the two very different forms of learning and memory. To begin examining this issue, we trained one group of animals in delayed nonmatching-to-sample (DNMS) with trial-unique stimuli, a measure of visual recognition, and another group in concurrent discrimination learning with 24-h ITIs (24-h ITI task), as the measure of habit formation. We then assessed the separate effects of scopolamine and haloperidol, a muscarinic cholinergic and a dopaminergic antagonist, respectively, on the animal's performance on each of these two tasks. The effects of systemic administration of two different doses (10 and 17.8 $\mu\text{g/kg}$) of each of these two drugs were compared against the effects of saline injections.

The subjects were six experimentally naïve monkeys (*Macaca mulatta*; five males, one female), ranging in weight from 5.2

to 8.0 kg. They were housed individually or in established pairs and fed a diet of primate chow (PMI Feeds) supplemented with fruit; water was available ad libitum. Three of the animals were trained on the visual recognition task and the other three (including the female) on the visual discrimination learning task. All procedures were carried out in accordance with both the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the principles described in the Declaration of Helsinki and were approved by the Animal Care and Use Committee of the National Institute of Mental Health.

Behavioral testing was conducted in sound-attenuating operant chambers equipped with 15-inch touchscreen monitors (Microtouch, 3M Center) programmed by LabView software (<http://www.ni.com/labview/>). Monkeys were seated in transport chairs designed to allow free arm movements. Correct choices were rewarded with pellets (190 mg, 50:50 mixture of banana and fruit punch flavors, Research Diets) dispensed into a metal cup located beneath the lower right corner of the touchscreen. Animals were initially habituated to the transport chair and operant chamber, given a few sessions of noncontingent access to food in the cup, and then gradually shaped to touch colored squares on the monitor to obtain the food pellets. This preliminary phase typically required a week, after which the two groups began training on their respective tasks.

The recognition memory group was first trained in the rule for DNMS with trial-unique stimuli. The stimuli were drawn from a 2000-item library consisting of clip art and digitized photos, each 10 cm² when displayed on the monitor. For each trial, a stimulus was selected randomly from the library and presented as the sample in the center of the monitor. Touching the sample resulted in reward delivery. This was followed 5 sec later by simultaneous presentation of the sample and a randomly selected novel stimulus, one appearing on the left and one on the right, 10 cm apart, with their left/right positions determined pseudorandomly. On this choice trial, touching the novel stimulus led to reward delivery. A daily session consisted of 60 such trials, each with a new pair of stimuli, and with the trials separated by 10-sec ITIs. Training continued until the animal achieved the criterion of 90% correct responses on three successive sessions. Following this rule learning, the list length, i.e., the number of sample stimuli to be remembered on each trial,

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was then gradually increased, as was the length of the intervals between successive samples in the list (ISIs) and the interval between successive choice tests (ICIs). The final task parameters were list lengths of 20 trial-unique stimuli, with both ISIs and ICIs of 20 sec each and with the sample stimuli presented in the same order on the choice tests as in the sample phase. This 20-trial block required the animal to recognize stimuli presented 8–10 min earlier. Four such blocks, separated by 20-sec interblock intervals, were presented daily for a total of 80 trials/session. The animals were trained on this final task to a level of 80% correct responses and then overtrained with and without systemic injections of saline (see below), before being evaluated with each of the two pharmacological agents.

The discrimination learning group was trained on sets of concurrent visual discriminations with 24-h ITIs. Each set consisted of 20 pairs of stimuli of the same types, and displayed on the monitor at the same size and distance apart, as those used in the choice phase of the recognition task. On the first day, the 20 pairs were shown just once each at 20-sec intervals, with one member of each pair arbitrarily designated as the positive one, i.e., leading to delivery of food reward when touched. The same 20 pairs were then re-presented daily in the same order, with the positive and negative items in each pair remaining the same as on the first day, and with only their left-right positions varying pseudorandomly across the daily sessions. Testing continued until animals achieved the criterion of 90% correct responses across three consecutive sessions, after which they received additional sets of 20 pairs, each composed of an entirely new set of stimuli. Once the animals established a consistent rate of learning new sets to criterion, which turned out to average ~11 sessions per set, the rate at which they learned new sets was evaluated under the effects of daily injections of either saline, scopolamine, or haloperidol, as well as in the noninjection control condition.

For three days prior to the start of saline and drug testing, animals were habituated, while off task, to the procedures associated with intramuscular injections, receiving sterile saline (pH 7.4, 0.1 mL/kg) on each of these days. On testing days, we administered either vehicle (sterile saline) or an ascending and descending series of two doses of each drug: 10.0 and 17.8 μ g/kg of the salt form of scopolamine HBr (Sigma-Aldrich), and 10.0 and 17.8 μ g/kg of haloperidol (Ortho-McNeil). These doses were selected on the basis of published data regarding scopolamine's effects on DNMS (Aigner and Mishkin 1986; Aigner et al. 1991) and unpublished pilot data gathered on other animals

tested with haloperidol on discrimination learning with 24-h ITIs. Each compound was dissolved in sterile saline and administered intramuscularly in a volume of 0.1 mL/kg 20 min prior to task onset. To block scopolamine's peripheral side effects, scopolamine injections were preceded 1 min earlier by injection of the identical dose of neostigmine methylsulfate (Sigma-Aldrich). The DNMS group was required to perform at least one saline session at criterion prior to a subsequent drug-testing session. The 24-h ITI group was required to learn a new 20-pair set at their

Figure 1. (A) DNMS scores are mean percent errors $\pm$ SE collected from six noninjection control sessions, six saline sessions, and two sessions per drug dose, one ascending and one descending. (B) Scores on the 24-h ITI task are mean number of sessions to criterion $\pm$ SE and mean errors to criterion per stimulus pair (depicted by symbol within each bar) collected in each monkey from 19 noninjection control sets, 17 saline sets, and two sets per drug dose, one ascending and one descending. Doses are in μ g/kg. (S) Scopolamine; (H) haloperidol.

previously established control rate of ~11 sessions preceding criterion before receiving the next set accompanied by daily drug injections.

The three animals trained by approximation on the DNMS task with list lengths of 20 took an average of 6200 trials from the start of training to attain stable performance of greater than 80% correct responses on these lists, which required them to recognize each of 20 stimuli presented just once 8–10 min earlier. The three animals trained on the 24-h ITI task took an average of 17 sets to reach a criterion of 90% correct responses at a consistent learning rate of 11 sessions per set, each set consisting of 20 pairs of stimuli presented just once per day.

The effects of the drugs on each task (Fig. 1) were first assessed with a one-way ANOVA (saline and each dose of each drug) for repeated measures, which showed significant differences in both (DNMS: $F = 26.28$, Greenhouse-Geisser corrected $df = 1.19, 2.38$, $P = 0.025$; discrimination learning: $F = 9.18$, Greenhouse-Geisser corrected $df = 2.12, 6.41$, $P = 0.013$). The ANOVAs were followed with two-tailed t -tests for related samples. Compared with the saline scores of the group trained on DNMS (mean percent error and standard error: 17 ± 1), both doses of scopolamine produced impairment in recognition accuracy (S10, 33 ± 3 , $P < 0.05$; S17.8, 43 ± 3 , $P < 0.01$), whereas neither dose of haloperidol had a significant effect (H10, 18 ± 1 , $P > 0.8$; H17.8, 19 ± 2 , $P > 0.1$). Conversely, relative to the saline scores of the group trained on the discrimination learning task (mean sessions to criterion and standard error: 8.4 ± 0.5), neither dose of scopolamine had a significant effect on learning rate (S10, 7.8 ± 0.9 , $P > 0.9$; S17.8, 12.0 ± 0.2 , $P > 0.2$), whereas both doses of haloperidol led to an impairment (H10, 26.8 ± 4 , $P < 0.05$; H17.8, 26.6 ± 3 , $P = 0.05$). Importantly, each dose of the effective drug on a given task impaired performance in comparison not only to saline but also to each dose of the ineffective drug on that task (all P s < 0.04).

This evidence implying a clean-cut double dissociation of deficits must be qualified, however, for at least two reasons. First,

an error analysis on the concurrent discrimination task (ANOVA followed by post-hoc t -tests) indicated that, whereas each haloperidol dose resulted in more errors to criterion than did either scopolamine dose (P s < 0.05), the higher dose of scopolamine also led to an increase in errors to criterion that fell just short of significance (saline, 2.45 ± 0.12 ; S17.8, 3.90 ± 0.25 ; $P = 0.058$), suggesting that this dose might well have yielded a small but significant retardation in rate of learning had our sample size been large enough to detect it. Second, the haloperidol-induced impairments on this task could have been due to the cumulative adverse effects of chronic drug administration on behavioral processes unrelated to habit formation per se. Although analysis of average daily scores (Fig. 2) indicates that this learning deficit was evident from the start, reaching significance from day 3 onward (P s < 0.05), the possibility cannot be ruled out that the deficit was prolonged by general behavioral interference resulting from repetitive and widespread dopamine receptor antagonism. (The smaller and shorter-lasting effect of scopolamine attained the 0.05 level of significance only on days 4 and 8.)

Even with these caveats, the differential effects of the two drugs on the two tasks are noteworthy, given that the rhinal cortical tissue serving recognition memory and the neostriatal tissue mediating discrimination habits can each be influenced by both neuromodulatory systems (e.g., Mesulam et al. 1986; Haber and Fudge 1997; Thomas et al. 2000; Alcantara et al. 2001; Lewis et al. 2001). Indeed, *in vitro* studies indicate that the two neuromodulators can interact within the same cells (e.g., Calabresi et al. 2000; Pisani et al. 2000; Suzuki et al. 2001; see also Reynolds et al. 2004).

Perhaps different task parameters from those used here, or the administration of different types of receptor antagonists, would lead to a different outcome. For example, the introduction in the DNMS task of delays much longer than 10 min might recruit mesocortical dopaminergic mechanisms (Huang and Kandel 1995) that were not drawn upon by the memory demands in this study; and the use of nicotinic rather than muscarinic antagonists (Wonnacott et al. 2000; Zhou et al. 2001; Partridge et al. 2002; Keath et al. 2007) might reveal a far greater participation of cholinergic mechanisms in the formation of discrimination habits.

Nevertheless, given the present task parameters and pharmacological agents, each of the two contrasting forms of learning—memory and habit—appears to depend primarily on a different one of the two neuromodulators that were manipulated, suggesting that these two neuromodulators—acetylcholine and dopamine—have contrasting effects on the modification of their associated synapses. This proposal is based on the following considerations.

Activation of cholinergic muscarinic receptors in rhinal cortex during just a single viewing of a novel stimulus apparently modifies the visuo-rhinal synapses by an amount sufficient to encode that stimulus in memory as a highly familiar one. This is indicated by the animal's ability to recognize that particular stimulus at better than 90% accuracy after a delay of 1–2 min (Gaffan 1974) and at a level of 80% accuracy even after a delay of about 10 min (this study). There is, however, a cost to such an effective

Figure 2. Scores are mean percent correct responses $\pm$ SE for each successive test session from day 1 through day 11. The saline scores are averaged across 17 sets/monkey, and the scores for each drug are averaged across four sets/monkey (two sets at each of the two doses, one ascending and one descending).

synaptic change that is so quickly and readily induced, namely, the modification is usually a transient one; recognition accuracy commonly decays to levels close to chance in less than an hour (Murray and Mishkin 1998). This, presumably, is the reason that the cholinergic-dependent visuo-rhinal circuit cannot mediate discrimination learning when delays between successive test trials last 24 h. Such long intertrial delays require long-lasting synaptic modification, and, indeed, it is the durability of the synaptic change induced by each activation of dopamine receptors in the visuo-striatal circuit that allows the effects of reward-prediction training to accumulate even when the training is presented at the rate of only a single trial per day. But there is a cost to this form of plasticity as well. Each trial-inducing synaptic modification may be long-lasting, but the change is a relatively small and ineffective one by itself, insufficient to increase visual discrimination performance by more than a few percentage points each time (see Fig. 2). Hence the need for several trials before the steady, day-by-day, accretion of synaptic changes increases the strength of each of the discrimination habits to a stable level of 90% correct responses. The possibility that the synaptic modifications induced by the two neuromodulators at their respective sites have these strikingly different properties calls out for direct cellular and molecular study.

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