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Pharmacology of Temporal Cognition in Two Mouse Strains*

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Behavioral and pharmacological testing in mice has been revamped following the development of new tools for the manipulation of genetic information. We present the results from the peak procedure, an operant test that assesses the capacity to perceive, remember, and act upon temporal information. We studied the basic timing abilities in two different strains of mice, the C57Bl/6J and C3H/HeJ, and their response to psychoactive substances. Scopolamine and high doses of d-amphetamine disrupted performance by increasing response variability. The effect of d-amphetamine was particularly clear in C3H mice. Whereas scopolamine did not seem to affect the location of the response, the effect of a low dose of d-amphetamine, a leftward shift, was consistent with the hypothesis that it accelerates the internal time keeping mechanism. Physostigmine alone improved performance by reducing variability between trials without affecting the response location. Pretreatment with physostigmine partially blocked the deleterious effects of scopolamine. Methylphenidate did not have major effects on timing behavior in C57 but in the highest dose shifted the response of C3H mice to the left. The higher sensitivity of the C3H strain to the effects of d-amphetamine and methylphenidate support its value as an animal model of attention deficit disorder. The performance of mice in this temporal task was comparable to that observed in rats and pigeons, and seemed exquisitely sensitive to pharmacological manipulation.

As a result of the advances in molecular biology and the new tools for genetic manipulation, a higher proportion of research in neuroscience is now focused on mice (see Nestler et al., 2001; Koob et al., 2001). A Medline search shows that, during the 1980s, about a third of the research in rodents was conducted with mice; while in the 1990s the proportion grew to about one half. This trend created a need to develop and optimize procedures that are standard in other species, for the behavioral and pharmacological characterization of novel knockout and transgenic mice. Several papers in this special issue address the development and validation in mice of a timing test, the peak interval (PI) procedure (Carvalho et al., 2001; King et al., 2001). This is also the main goal of the present article.

The ability to perceive, encode, and act upon temporal information is central to many daily activities. Animals are able to anticipate events in temporal patterns that occur in a range from seconds to hours, and to adapt to cyclic

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environmental changes in a range from hours to days. Humans, rats, pigeons and starlings (Church et al., 1976; Kacelnik et al., 1990; Wearden & McShane, 1988) have been used to study the psychophysics of time perception, although the rat has been the species of choice for the study of its neurobiology (Meck et al., 1984). The present article provides pharmacological data using the PI procedure in two different mice strains, C57B/6J and C3H/HeJ, both from Jackson Labs.

The PI procedure was developed by Catania (1970), Roberts (1981), and more thoroughly by Gibbon and his colleagues (Church et al., 1994; Gibbon & Church, 1992). It is a psychophysical task based on the perception, memory, and reproduction of temporal information. In the PI procedure, animals are trained to work for food reinforcement that is delivered at the same time during each trial (fixed interval or FI); food is withdrawn during some subsequent nonreinforced trials. Whereas in a standard FI procedure, the rising response rate (FI scallop) is truncated at the time of reinforcement, in the PI procedure the response rate increases up to a maximum at the scheduled time of reinforcement, and then decreases, providing not only a scalloped shape but also a peak of responding (peak time). The peak time and the shape of the response rate curve indicate whether the animal is sensitive to the time of reinforcement. To perform well in this task, animals need to learn an association between a response (lever pressing, nose poking or key pecking) and the delivery of reinforcement, perceive and remember time, act on the remembered time by responding or by inhibiting a response, and compare the elapsed time during a trial with their memory for the time of reinforcement.

Few timing studies have used mice as subjects. The first published report of timing in mice, (woodmice; Lejeune & Wearden, 1991), used the FI procedure. Later, Brunner and Hen (1997) studied temporal information processing in the context of choice between rewards in serotonin-receptor mutant mice. We review Lejeune and Wearden's (1991) data as a way of pointing out the complexities of temporal procedures and interpretation of the data. A thorough review of the timing literature can be found elsewhere (e.g., Allan, 1979; Meck, 1996; Paule et al., 1999).

Figure 1 shows the woodmice's responding under four different FI schedules: 60, 120, 180, and 240 s. Woodmice clearly had no difficulty in timing the reinforcement as shown by a sharp scalloped response curve with a consistent increase in responding from zero, at the beginning of the trial, to the highest level near the appropriate reinforcement time (Figure 1a). Another interesting characteristic of temporal responding is exemplified by these data, as explained below.

In timing tasks such as the FI and PI procedures, it has been proposed that animals estimate time using an internal clock that can be started at the beginning of a trial and reset by reinforcement (Treisman, 1963). Responding reaches a maximum as trial time elapses and the estimate in the internal clock matches the remembered reinforcement time. The peak of the response curve marks the maximal expectancy for reinforcement (temporal accuracy) and the sharpness of the increase and decrease in response rate marks the acuity of the temporal memory. As in other psychophysical tasks, some general laws have been proposed and supported by empirical studies. For example, as the fixed interval is increased, the peak time also increases, an expected result for an animal with good short- and

long-term memory. The position of the peak, however, varies from trial to trial, and this variation (to be exact, its standard deviation) is proportional to the FI value (i.e., Weber's law for time perception). An extension of Weber's law also suggests that the spread of the response curve should also be proportional to the FI (i.e., the longer the FI, the flatter the response curve). Therefore, response rates plotted on a relative time axis (in units of a fraction of the FI) should overlap.

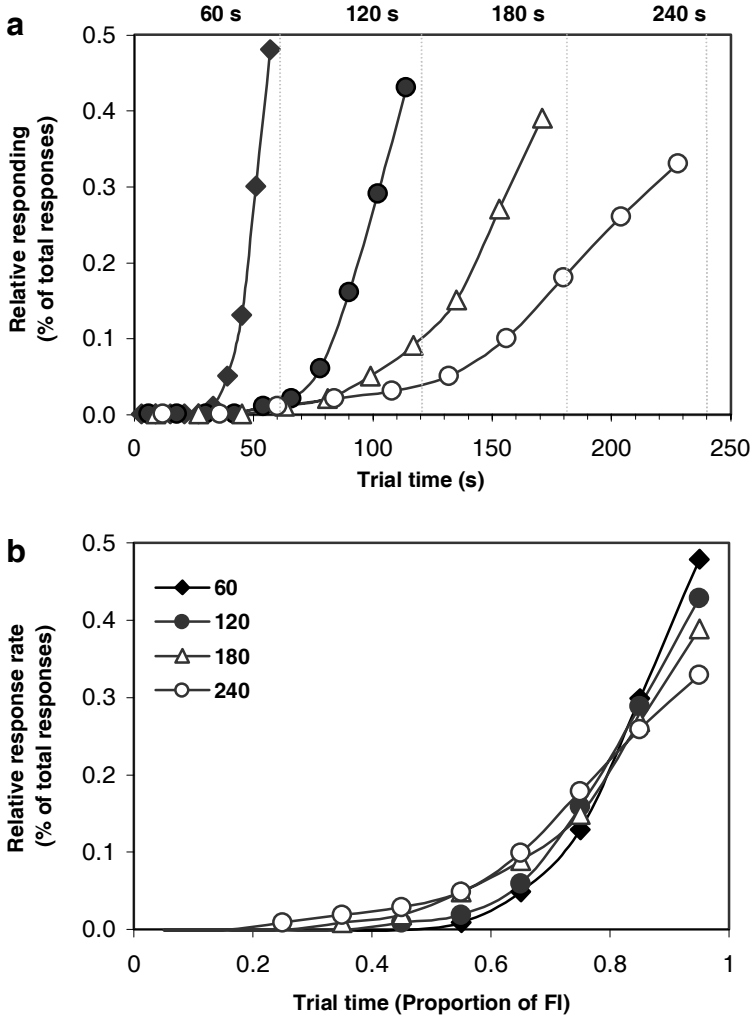

Figure 1. Relative responding under different FI schedules (A), and percent of total responses per session (B) of woodmice plotted as a function of trial time. From Lejeune and Wearden (1991). Copyright Academic Press, reproduced with permission.

Figure 1b shows that the relative responses rates from woodmice (Lejeune & Wearden, 1991) do overlap on a relative time scale although some systematic departures seem to be present, as the response curves generated by longer FIs seem to be flatter, even on a relative time scale. Lejeune and Wearden (1991) argued that such discrepancies might be explained as the result of a non-attentional mechanism

that overlaps with pure, Weberian timing, a possibility that is intriguing and worth investigating.

Weber's law points to the difficulty animals have in distinguishing a signal from its surrounding informational noise. In the FI 30 s, for example, animals should inhibit responding at the beginning of a trial as reinforcement is never delivered before 30 s. How easy is it for an animal to distinguish between 0 s and 30 s? Probably very easy, as response rates at zero time are very low. But as time elapses and nears the scheduled reinforcement time, the discrimination becomes more difficult. Weber's law says that although the discrimination between 10 s and 30 s, is easier than that between 20 s and 30 s, it is as difficult as the discrimination between 100 s and 300 s. In other words, if the ratio of the magnitude between two stimuli is the same, the difficulty of the discrimination should also be the same.

As time elapses during a trial, the estimate of the current time and the remembered time of reinforcement grow more similar and, therefore, more difficult to differentiate. This difficulty is then manifested as an increase in response rate. Whereas an animal with perfect timing sense will show a step-function response (i.e., no response until reinforcement time, then responding at the time of reinforcement, and no responding afterwards), a normal animal will show a noisy response curve that increases before reinforcement time and decreases afterward.

Temporal tasks are like signal detection tasks: sensitive to the saliency of the stimulus and to the attention it draws. Poor attention results in a low signal-to-noise ratio and, therefore, impaired performance. Attentional dysfunctions can be detected by measuring responding (an animal that is unable to attend to temporal information will have a flat response curve) and by looking at response variability, a hallmark of attentional dysfunction (as attention weaves in and out, performance varies from trial to trial).

The complex data produced by the peak procedure not only provides a measure of memory content and memory strength but also provides a sensitive pharmacological tool. Any change that brings responding closer to the performance of a perfect timer would be considered an improvement in timing. A true cognitive enhancer therefore should decrease responding before and after reinforcement time, without affecting the peak time (apart from making it more sharply attuned to reinforcement time). Any drug that produces nonspecific locomotion activation, sedation, or toxicity will result in impaired (flatter) performance.

Drugs that enhance cholinergic activity, such as the cholinesterase inhibitor physostigmine, seem to both enhance and speed up performance (Meck & Church, 1983), by sharpening the curve and by inducing a leftward shift in the response curve. Cholinergic antagonists, such as the muscarinic receptor blocker atropine, impair or slow down performance (Meck & Church, 1983), inducing a flattening and a rightward-shift of the curve. Indirect dopaminergic agonists, such as methamphetamine or d-amphetamine, also act by enhancing or speeding-up responding (Maricq et al., 1981), whereas dopaminergic antagonists, such as pimozide, slow down responding (Meck, 1986), although some studies have failed to find robust effects (Frederick & Allen, 1996). Different explanations have been given for such leftward or rightward shifts in the response curve (e.g., Meck, 1996; but see Chiang et al., 2000). Some have favored explanations using an internal

clock model arising from Treisman's original hypotheses (Treisman, 1963), whereas other nontiming processes have also been proposed as mediators of the dopaminergic antagonist action in chronic treatment experiments (Ohyama et al., 2000).

In terms of the clock model, Meck and Church (1987a; Meck, 1996) have argued that amphetamine and other similar drugs accelerate the rate of the internal pacemaker that forms the basis of the timing apparatus. As the pacemaker ticks faster after an acute injection of the drug, time seems to elapse faster, the response curve shifts to the left, and the peak response time occurs earlier than it does under baseline conditions. This also implies that under the drug effect, during reinforced trials, reinforcement comes later than expected.

In this paper we report the effects of the dopaminergic drug d-amphetamine, the amphetamine-like methylphenidate, and the cholinergic drugs scopolamine and physostigmine. We concentrate on two aspects of performance: First, relative response rates were used to assess whether a drug sharpened or flattened response curves. Second, the position and variability of the peak response time were used to assess whether the drugs affected temporal accuracy and acuity. Strains of mice differ greatly in behavior and physiology, and therefore conclusions regarding drug effects and temporal or cognitive abilities need to be based on studies comprising several strains. Most studies focusing on comparisons between mouse strains report large difference in both spontaneous and drug-induced behavior.

In this article we report the timing performance of two common strains of mice, C57Bl/6J and C3H/HeJ (Jackson Labs; thereafter referred to as C57 and C3H) that have been repeatedly shown to differ in behavior and in their response to pharmacological manipulations (Helmeste & Seeman, 1982; Kuribara & Tadokoro, 1987). For example, the two strains under study have been shown to differ greatly in dopamine D2 receptor binding, with the C3H strain showing higher receptor density than the C57 and higher sensitivity to the hypolocomotor effects of low doses of d-amphetamine (although both strains show similar locomotor stimulant effects to high doses of this drug; Helmeste & Seeman, 1982). C3H mice have also been shown to be especially sensitive to scopolamine (Kuribara & Tadokoro, 1987). We chose the C57 and the C3H strain also because they are common strains for genetic studies (e.g., Marks et al., 1986), and are also routinely used as genetic backgrounds for transgenics and knockouts (C57; see http://tbase.jax.org/) and for spontaneous mutations (C3H; see http://www.jax.org/). We presently characterize the response of the C3H strain to scopolamine and physostigmine in a temporal task and, in the last two studies we used low doses of methylphenidate and d-amphetamine to explore the potential differential sensitivity of these two strains.

Method

Subjects

Subjects were 13 naive male C57B/6 (C57) and two groups of naive male C3H/HeJ (C3H) mice (Jackson Labs), approximately 8 weeks of age when the experiments started. One group of 15 C3H was used for the study comparing acquisition between strains, the effects of scopolamine and physostigmine, and methylphenidate. A different group of 32 C3H mice was used to assess the

effects of d-amphetamine. Mice were kept on a 12:12 h light:dark cycle (lights on at 07:00 h), and maintained at 22 °C (±2 °C) and 60% humidity. Subjects were individually housed and trained in the same experimental room. All mice were food deprived to a target weight of 85–90% of their free-feeding weight before training began. Mice were fed approximately 10% of their body weight until they reached their target weight. On average, 1 week of food deprivation was sufficient to reach the target weight. During this time, all subjects were fed Bioserve 500 mg precision dustless pellets as their daily ration. They were exposed to Carnation™ evaporated milk in the home cage to avoid a possible neophobic reaction to the reinforcement. Subjects were given 1-week "vacations" every 4 to 6 weeks at which time they were allowed free access to food and a new free-feeding body weight baseline was recorded. Water was continuously available in the cage.

Apparatus

Sixteen mouse operant chambers (MED Associates, Vermount, U.S.A.) were configured identically with two retractable ultra-sensitive levers (although only the right lever was used for these experiments), stimulus lights, and a dipper for the distribution of the condensed milk reinforcement. A house light was positioned on the opposite wall from the lever. Each chamber was located within an attenuating cubicle, which was equipped with a fan to help mask peripheral noise as well as provide adequate circulation of air.

Procedure

Training. Training and testing consisted of 1-h daily sessions. Subjects started on a concurrent fixed ratio 1, fixed time 1 min (FR 1 FT 1 min) schedule of reinforcement in which the house light served as the discriminative stimulus. Food was delivered every 1 min but the delivery was immediate if the animal made a response. Most animals acquired the lever-press response quickly, and those that did not were manually shaped by reinforcing successively closer approximations to the dipper using pinhole video cameras mounted in the attenuating cubicles.

After no more than 1 week on this schedule, mice began training with a FI 10 s schedule in which all trials were separated by a 20 s, intertrial interval. The house light was on during the FI but off during the intertrial interval. Once a scalloped response curve was achieved, all subjects were placed on an FI 30 s schedule for approximately 1 week before moving to the PI procedure.

Testing. Peak trials were programmed to occur at random with the restriction that no more than two nonreinforced trials be presented consecutively. In peak trials, the house light was presented for 120 s. There was an average of eight peak trials per session. Responding was recorded in bins of 5 s and monitored graphically. When the response rate showed a clear peak centered at 30 s, subjects were considered well trained. On average, it took 12 days for the mice to reach a clear peak. Animals were considered ready for pharmacology when responding clearly increased before 30 s had elapsed and then decreased afterwards.

Drug Preparation and Administration. All drugs were dissolved in physiological saline and administered i.p. at a dosing volume of 10 ml/kg. At least 2 days were allowed between doses as a washing-out period. Drug testing was done twice a week, with normal training (no injections) during the remaining 3 days. ***Scopolamine and physostigmine:*** Saline, scopolamine (2 mg/kg, i.p.), and physostigmine (0.6 mg/kg, i.p.) were injected following four different treatments in a Latin-square design. All treatments comprised two injections, 60 and 30 min, respectively, before testing. The four treatments were: saline-scopolamine (SAL-SCO), physostigmine-saline (PHY-SAL), physostigmine-scopolamine (PHYS-SCO) and saline-saline (SAL-SAL). ***Methylphenidate:*** Saline or methylphenidate (Sigma, 1, 2, or 4 mg/kg) was administered i.p. in a volume of 10 ml/kg, according to a Latin-square design. ***D-amphetamine:*** Mice were treated with either vehicle or d-amphetamine (1, 2, or 4 mg/kg, i.p., in a volume of 10 ml/kg) in a Latin-square design.

Data analysis. To analyze temporal performance it is standard practice to look at the relative response rate, instead of the absolute rate (Brunner et al., 1996). The relative response rate is obtained by dividing each subject's absolute response rate by their maximum response rate and then multiplying by 100. For example, if an animal responded 10 times in the first bin (up to 5 s into the

trial), then 20 times (achieving its maximum responding) in the second bin (from 5 to 10 s into the trial) and then zero times in the rest of the trial, the relative response rate will be calculated as: 10 / 20 x 100 = 50% for the first bin, 20 / 20 x 100 = 100% for the second bin (where response rate was maximum) and 0 / 20 x 100 = 0% for the rest of the trial. This transformation eliminates individual differences in response rates and focuses the analysis on the shape of the response curve.

The start-stop analysis fits five horizontal segments to the response data from each peak trial of each subject. Although either three (low-high-low or LHL) or five segments (low-high-low-high-low or LHLHL) have been used in the past to fit data from the peak procedure (e.g., Brunner et al., 1997), in cases such as the present studies, more than one burst of responding is expected to appear in a considerable number of trials trial. Brunner et al. (1997) showed that the position of the first burst of responding is not affected by the existence of a second peak later in the same trial. As with three segments it is impossible to find a good fit to a trial characterized by such double responding (and spurious late peak times can be found by fitting a misleading model), allowing a second peak with a five-segment analysis is necessary. To avoid spurious fitting we imposed several restrictions on the parameters. The requirements were that the first, third, and fifth segments be lower than the second and forth, and that all segments were of 10 s duration or longer. This resulted in a LHLHL pattern. We also loosely required that the first high segment be close to the reinforcement time (start < 40 s and stop < 85 s). If the second response burst was found to start before 60 s, it was not considered as a true second peak, but as part of the first (and unique) high segment. This resulted in two clearly distinct peaks being required for a two-peak fit, otherwise the procedure resulted in a simple single peak analysis, or a LHL pattern.

To find the best fit, each trial is partitioned in the five segments fitting the described requirements. Given a partition, a candidate model is selected by computing a response plateau in each segment as the average of the responses in that segment. Of all the candidate models (corresponding to all possible valid partitions), with plateau levels matching the pattern of LHLHL (i.e., the first plateau is lower than the second which is higher than the third, etc.), we choose the model for which total variance is minimal. In a negligible number of cases, there was no candidate model that matched the LHLHL pattern, and those cases were rejected. (The program was designed by Dr. G. Stolovitzky and is available upon request.) We included all other trials in the analysis independently of their goodness of fit. The peak time was calculated as the middle of the first high segment and the spread of the response burst was its length. As a measure of variability between trials we calculated the standard error of each of the four measures (start, stop, peak time, and spread) for each mouse in each condition. Analyses of variance were carried out on all the data using strain as between-subject factor and training day, treatment, and trial time as a repeated-measure factors whenever appropriate. Significance is reported at alpha = 0.05.

Results

Strain Differences

There were clear strain differences in the acquisition of the peak procedure, during the first 10 days after the introduction of peak trials (Figure 2). While both strains acquired the task quickly, the C57 strain showed steady improvement over the first 10 days of PI training (where “improvement” means a sharpening of the peak around the reinforcement time) whereas the C3H mice did not show great changes in performance during the same period: Day x Strain interaction: $F(3, 78) = 5.81$. The shape of the response curves was also different: Day x Strain x Trial Time interaction: $F(69, 1794) = 1.75$, especially on Day 10. On this last day, C57 responded more, in relative terms, at the beginning of each trial but were better at inhibiting their responding after the expected time of reinforcement. This was confirmed with separate analyses looking at strain differences before (0-20 s into the trial) and after reinforcement (35-55 s into the trial); main Strain effect: $F(1, 26) = 6.98$ and $F(1, 26) = 10.00$, respectively.

The data indicated that, despite their differences, both strains are suitable for pharmacological manipulation. Although the first study we report is a large

study conducted in C3Hs only, we also report studies with both strains tested under both methylphenidate and d-amphetamine.

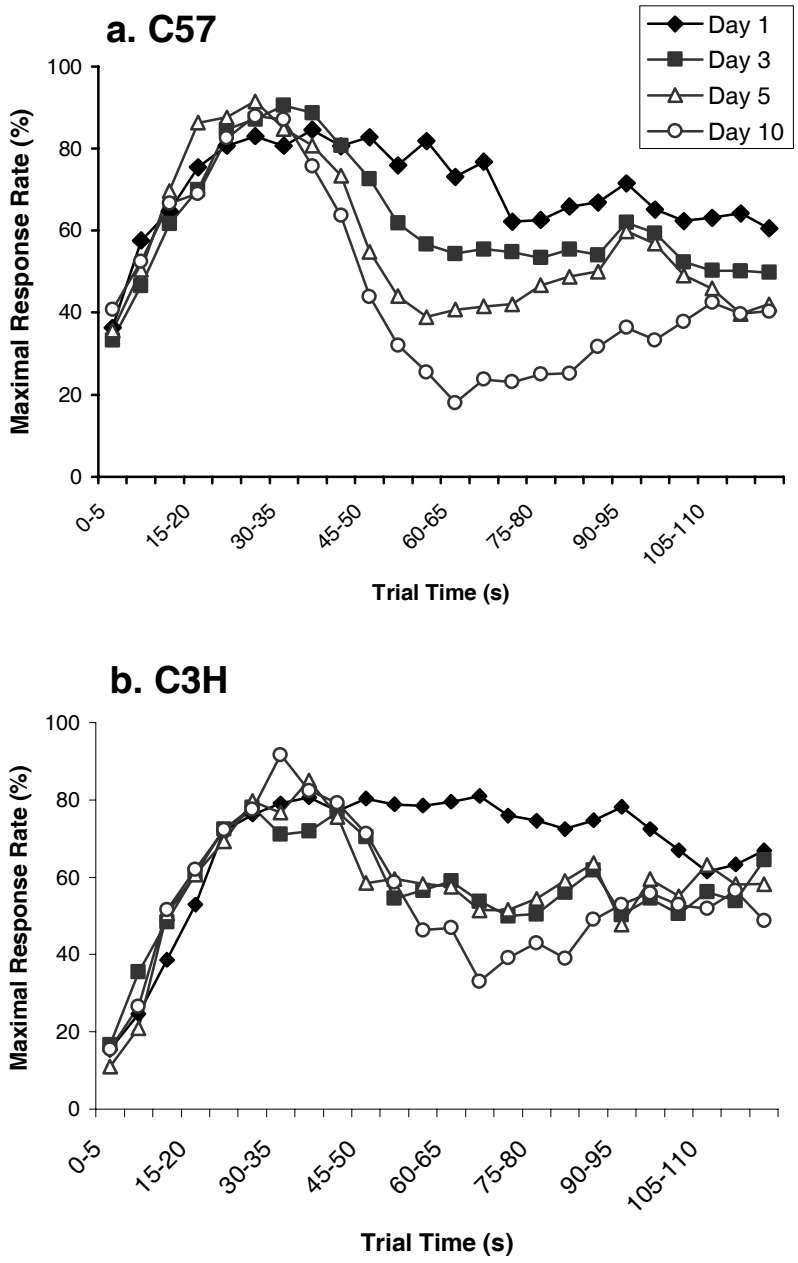

Figure 2. Mean ± standard error (SE) relative response rate as a function of trial time in C57 (A) and C3H (B) mice in peak trials across training days.

Response to Scopolamine and Physostigmine in C3H

We challenged mice with scopolamine to explore the sensitivity of the C3H strain to cholinergic manipulation in a temporal task. We also pretreated them

with physostigmine, in a different condition, to assess physostigmine's ability to block possible scopolamine effects. Finally, we also injected the drug alone to explore possible enhancing effects of physostigmine.

Drug treatment affected the absolute response rate: main Drug Treatment effect: $F(3, 24) = 3.63$. In particular scopolamine decreased response rate and physostigmine blocked its effect (planned comparisons: SAL-SAL vs. SAL-SCO and SAL-SCO vs. PHY-SCO: Fs$(1, 24) > 4.89$). Physostigmine had no effect alone.

To focus on the shape of the response curve, we then analyzed relative response rates. An ANOVA revealed large differences between the drug treatments in relative responding (Figure 3; Drug Treatment main effect: $F(3, 24) = 23.16$). Differences were mainly due to the effect of scopolamine, which impaired responding by flattening the response curve as compared with the saline-injected group: SAL-SCO vs. SAL-SAL planned comparison: $F(1, 24) = 28.14$. The effect of scopolamine on relative responding was apparently not blocked by a physostigmine pretreatment: PHY-SCO vs. SAL-SCO planned comparison: $F < 1$. Physostigmine alone did not have an effect on the relative responding as compared to the saline-treated group: PHY-SAL vs. SAL-SAL planned comparison: $F(1, 24) < 1.01$.

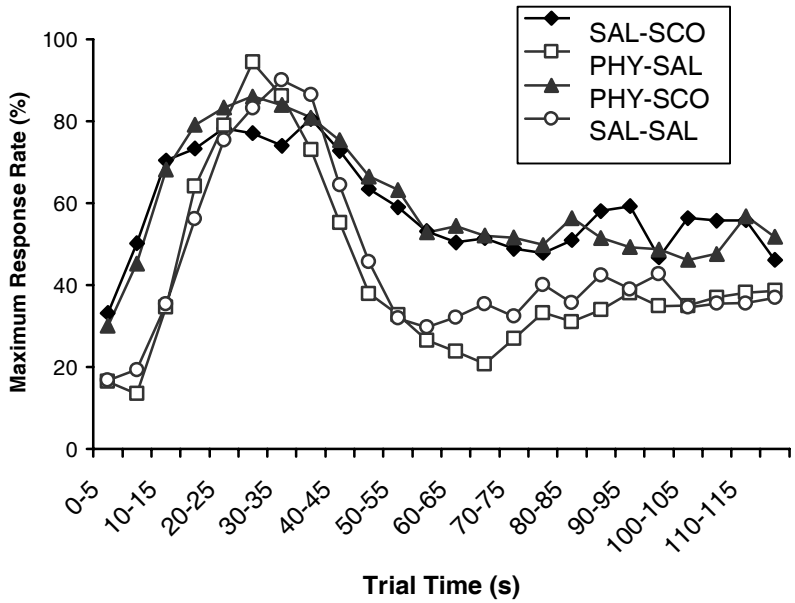

Figure 3. The effects of a vehicle, scopolamine (2 mg/kg) and/or physostigmine (0.6 mg/kg) injection on mean ± SE relative response rate in C3H mice as a function of trial time. All injections were i.p. with scopolamine being administered 30 min before the session, and physostigmine administered 60 min before the session. Subjects received an injection 60 min before each session of vehicle or physostigmine, and a second injection 30 min before the session of vehicle or scopolamine.

Response to Methylphenidate in C3H and C57

Methylphenidate did not have a significant effect on absolute or relative responding in either strain (Figure 4): Main Drug Treatment effects for relative

responding: *F*s(3, 39) < 1.60; Drug Treatment x Trial Time interactions: *F*s(69, 897) < 1.05. We conducted a series of planned comparisons at different time points during the trials to look for minor differences between the saline treatment and the difference doses, differences that may not be picked up by the overall analysis. In the C3H we found that only the 4 mg/kg dose induced a significant faster decay of the response at 40 s, consistent with a slight leftward shift (planned comparisons: $F(1, 24) = 5.47$). In the C57, this dose induced a faster raise of the response curve before reinforcement time, at 20 s, also consistent with a minor leftward shift, $F(1, 24) = 4.13$.

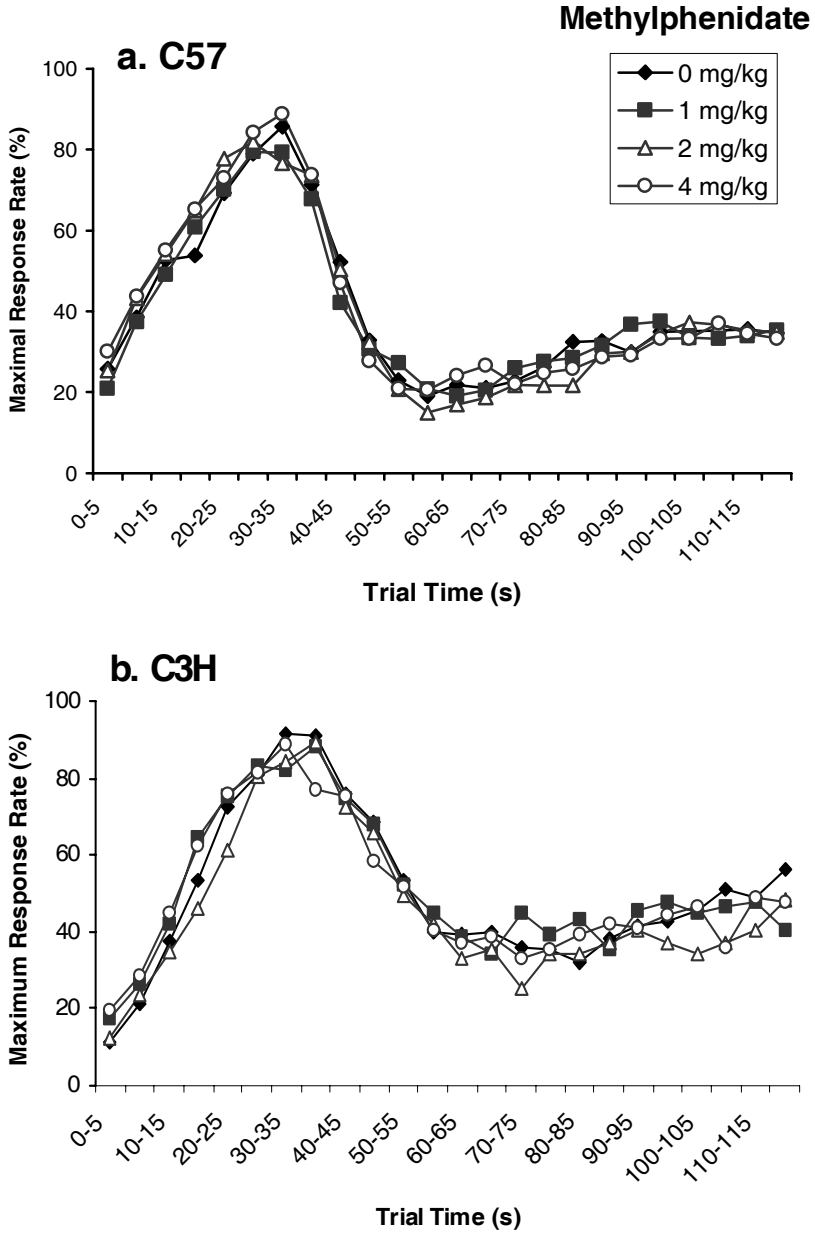

Figure 4. The effects of methylphenidate (administered i.p., 30 min before the session) on mean (± SE) relative response rate in C57s (A) and C3Hs (B) as a function of trial time.

Response to D-Amphetamine in C3H and C57

D-amphetamine decreased the absolute response rate in both C57 and C3H mice in a dose dependent manner, *F*s(3, 45) > 3.09. Whereas the effect was significant in the C3H mice for both the medium and the highest dose: planned comparisons, SAL vs. 2 and 4 mg/kg: *F*s(1, 45) > 6.69; in the C57 mice it only reached significance for the highest dose, *F*(1, 36) = 4.31.

To focus on the shape of the response curve independently of the overall levels of responding, we analyzed the relative responding. In C57 mice, an ANOVA found a non significant overall effect of d-amphetamine treatment but a significant effect of the drug treatment on the shape of the response curve (Figure 5a): Drug Treatment x Trial Time interaction: *F*(69, 828) = 2.43. Although vehicle-treated mice displayed a clear peak in responding at the expected reinforcement time, both the low and the medium doses of d-amphetamine were able to further improve performance. This is shown by a sharper peak resulting from a slightly (although not significantly) higher responding rate at the time of reinforcement and a significantly deeper decrease in responding after the reinforcement time. The response rate under the lowest dose of d-amphetamine was significantly lower shortly after reinforcement time: Planned comparisons between 1 mg/kg vs. saline: *F*s(1, 828) > 4.28, for trial times 55 and 60. The medium dose of d-amphetamine produced a similar pattern, with significantly lower responding after reinforcement time (planned comparisons between 2 mg/kg vs. saline: *F*s(1, 828) > 4.18 for trial times 50, 55, 60, and 65 s). The highest dose of d-amphetamine, 4 mg/kg, also significantly differed from the vehicle, but its effect was to impair performance as it flattened the response curve after reinforcement time: Planned comparisons between 4 mg vs. saline: *F*s(1, 828) > 4.22, for trial times 45, 70, 80, 85, 90, 95, 115, and 120 s.

D-amphetamine had a similar effect in C3H mice. It also produced an U-shape type of effect with the lower dose sharpening the response curve at about the time of reinforcement (Figure 5b): Drug Treatment main effect, *F*(3, 45) = 4.48; Drug Treatment x Trial Time interaction, *F*(69, 1035) = 2.87. The response rate with the lowest dose of d-amphetamine was significantly higher before reinforcement: Planned comparisons between 1 mg/kg vs. saline: *F*s(1, 1035) > 4.70, for trial times 10, 15 and 20 s. Response rate was lower after reinforcement time, although the difference reached significance only at 70 s into the trial: Planned comparisons, *F*(1, 1035) = 4.78. The medium dose of d-amphetamine flattened the response curve with significantly higher responding after reinforcement time: Planned comparisons between 2 mg/kg vs. saline, *F*s(1, 1035) > 4.19, for trial times 55, 60, 80 and 85 s. The highest dose of d-amphetamine, 4 mg/kg, produced a similar flattened response curve before and after reinforcement time: Planned comparisons between 4 mg vs. saline, *F*s(1, 1035) > 5.49, for trial times 10, 50-95, and 105 s.

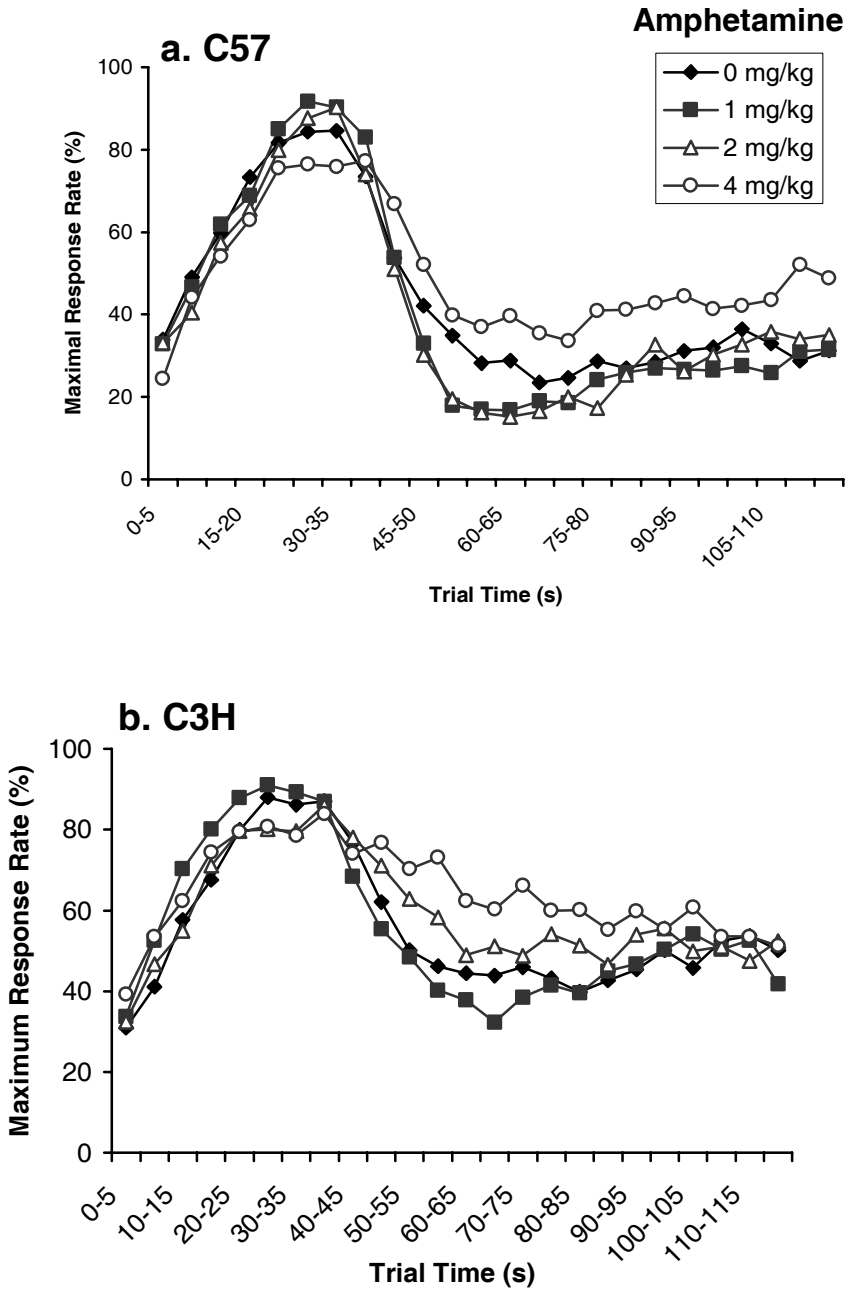

Figure 5. The effects of d-amphetamine (administered i.p., 30 min before the session) on mean (± SE) relative response rate in C57 (A) and C3H (B) mice as a function of trial time.

Trial-by-Trial Analysis

The first analysis removed performance effects by focusing on relative responding. This transformation focuses the analysis on the temporal location of the maximum responding and on the sharpness of the response curve. Looking at

average individual relative response curves, however, may obscure other effects as Figure 6 illustrates with hypothetical data. In Figure 6a, bursts of responding that are more of less centered at the same time, start and end at different times every trial. In Figure 6b bursts of similar spread happen at different times during the trial. Both hypothetical response patterns may show the same average relative responding, the same average peak time, start, stop and spread of responding. What differentiates both patterns is the variability between trials of certain measures. For both responding patterns the variability of the start and stop is similar but the variability of the spread is large in Figure 6a, but small in Figure 6b. Conversely, the variability of the peak time is small in Figure 6a, but large in Figure 6b.

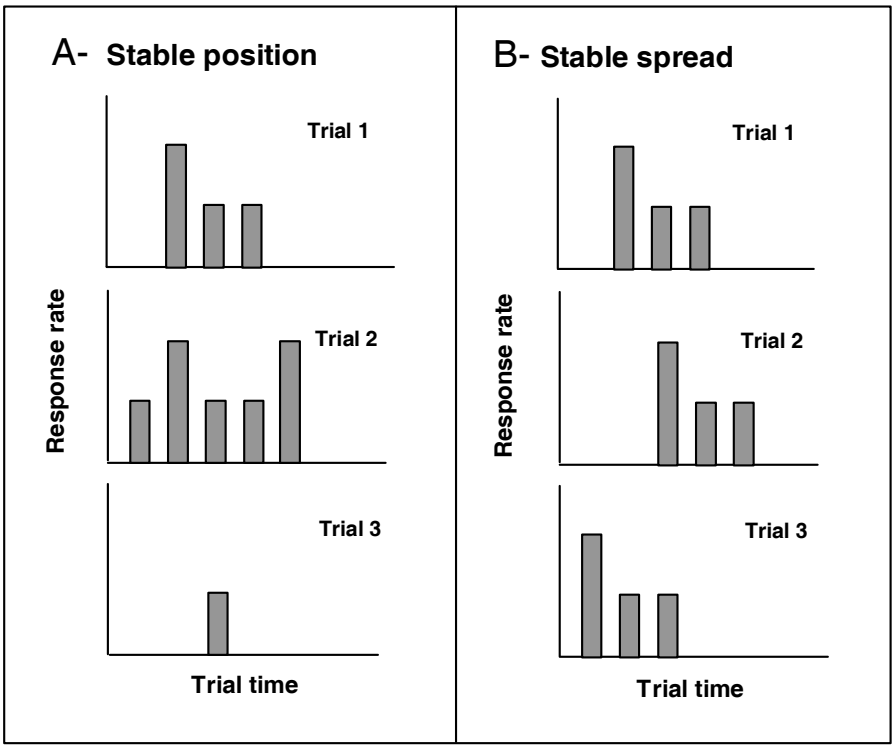

Figure 6. Three hypothetical individual trials showing a response pattern centered a similar average peak time with high variability in the duration of the response burst (A), compared against a pattern of consistent bursts durations with a highly variable peak time (B).

Figure 7 shows an example of a peak trial analyzed with the start-stop fitting procedure. The horizontal lines superimposed on the response rate show the best fit of the start-stop analysis routine with the resulting best five segments describing both the main burst of responding around reinforcement time and a second response burst towards the end of the trial.

The start-stop analysis has been used in the context of the scalar expectancy theory with the goal of supporting or disproving hypotheses derived from models of temporal information processing (Gibbon & Church, 1990). Here we use the trial-by-trial fitting procedure to extract information that, as discussed, may be obscured by averaging data from individual trials. It is assumed that a

cognitive enhancer would improve both the accuracy and the acuity of the temporal response. Accuracy will be measured mainly by the average position of the peak, and acuity will be measured by both the average spread of the distribution and by the trial-by-trial variability of responding. Thus a good cognitive enhancer may shift the peak time from a time later than the reinforcement time to the exact reinforcement time, reduce the spread of the response burst, and reduce the variability of both the peak and the spread. Conversely, any drug that produces the opposite effects will be deleterious. A drug that sharpens the response curve but shifts it excessively to the left (i.e., the peak time occurs too early) cannot be described as improving performance. Thus, if the peak of responding is shifted away from the time of reinforcement, then the effects may be due to an accelerated internal clock or pacemaker (Maricq et al., 1981).

We investigated next the effects of scopolamine, physostigmine, methylphenidate and d-amphetamine on temporal performance using the start-stop method of analysis.

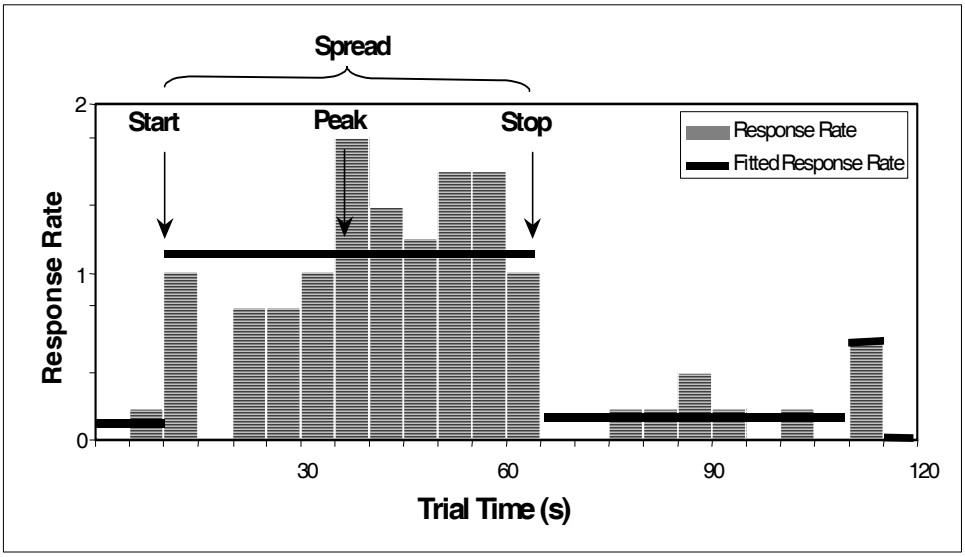

Figure 7. A trial-by-trial start-stop analysis on real mouse data illustrating how each trial is separated into five segments. The start, stop, peak time and spread of the first high segment estimate the response burst temporal location, duration and inter-trial variability.

Response to Scopolamine and Physostigmine. Figure 8 shows the mean and standard error of the four measures obtained from the trial-by-trial analysis in C3H mice: the start, stop, peak and spread values. This information is somewhat redundant because the parameters truly derived from the data are the start and stop scores, whereas the peak and spread scores are derived from them. The peak and spread are presented, however, because they more readily portray the performance of mice in every trial.

The drug treatment had no major effect on the location of the burst of responding, apart from a nonsignificant decrease in the peak and spread caused by physostigmine. The effect of physostigmine on the variability of the response

bursts between trials was significant as shown by a decreased in the variability of the spread, caused by a decrease in variability in the start, stop time and spread of the response burst: Planned comparisons, SAL-SAL vs. PHY-SAL, for start, stop and spread, *F*s(1, 24) > 4.39. Scopolamine had no effect on the temporal position of the response burst but increased the variability of the peak and stop: Planned comparisons, SAL-SAL vs. SAL-SCO, for peak, stop and spread, *F*s(1, 24) > 4.99. Pretreatment with physostigmine did not block the increased variability caused by scopolamine.

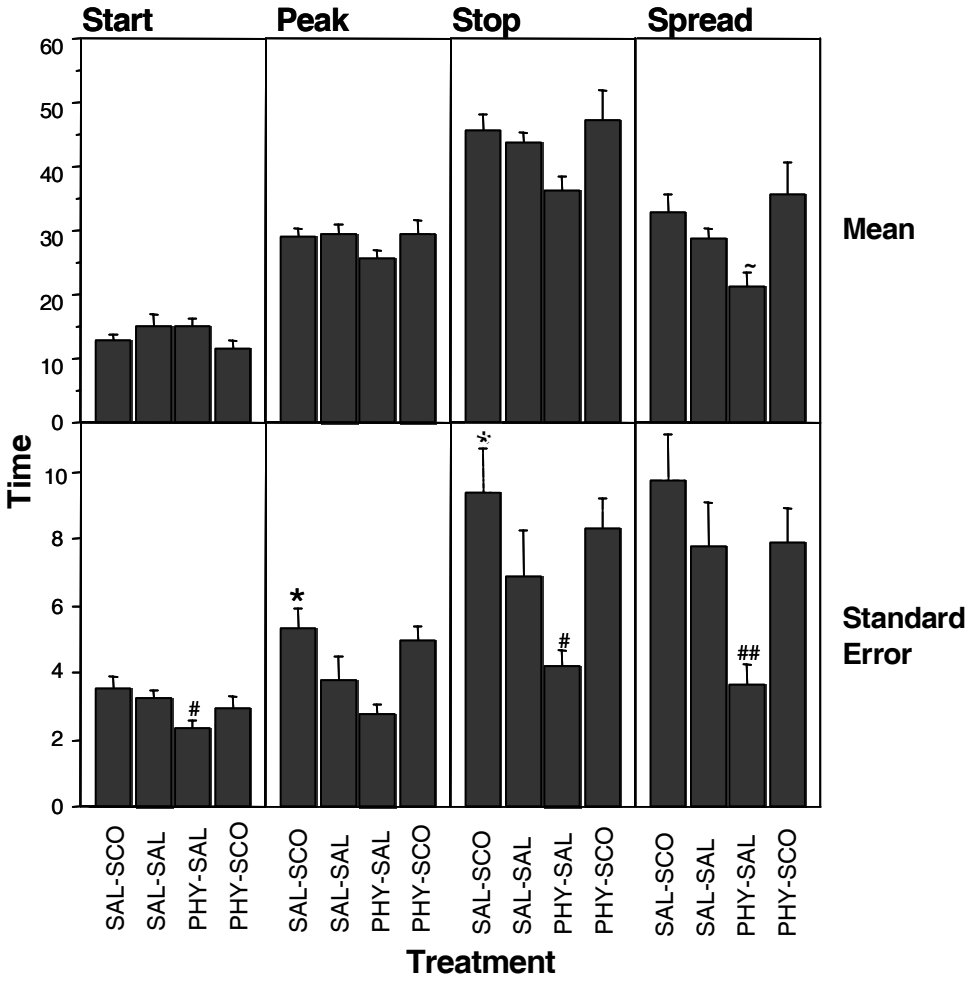

Figure 8. Mean (top) and standard error (bottom) of the four trial-by-trial parameters for C3H mice treated with scopolamine and/or physostigmine and a saline-injected control group. Numeral symbols indicate significant differences between the PHY-SAL and SAL-SAL treatment groups (#: $p < 0.05$, ##: $p < 0.01$). Asterisks indicate significant differences between SAL-SCO and SAL-SAL treatment groups (*: $p < 0.05$). ~: indicates a marginally significant effect ($0.10 < p < 0.05$)

Response to Methylphenidate in C3H and C57. Analyses of variance using strain and dose as factors, did not reveal any major main effect or interactions (data not shown). Planned comparison between the saline treatment and the three different doses, however, confirmed that the highest dose of methylphenidate shifted the response peak to the left in C3H but not in C57: Planned comparisons, saline vs. 4 mg/kg for peak: $F(1, 39) = 6.55$ and $F < 1$, for C3H and C57, respectively. The shift in the C3H represented a reduction of about 12% of the peak value (from 32.3 s to 28.3 s) and was purely due to a shortening of the stop value and not of the start value: Planned comparison, saline vs. 4 mg/kg for stop: $F(1, 39) = 5.05$. Methylphenidate did not affect measures of variability.

Response to D-Amphetamine in C3H and C57. Data from the two strains was analyzed next to assess any differential effect of the drug on the location of the response burst (Figure 9a) and on a measure of variability, the standard error, for each of these four measures (Figure 9b). D-amphetamine had no effect on the mean start time or its variability in either strain. The mean peak time, stop and spread were decreased by the drug, Fs(3, 81) > 3.09), to an equal extent in both strains. As the start was somehow fixed at the beginning of the trial, both these effects can be explained by a reduction in the mean stop times which "pushed" the peak time towards shorter times and reduced the spread of the response bursts.

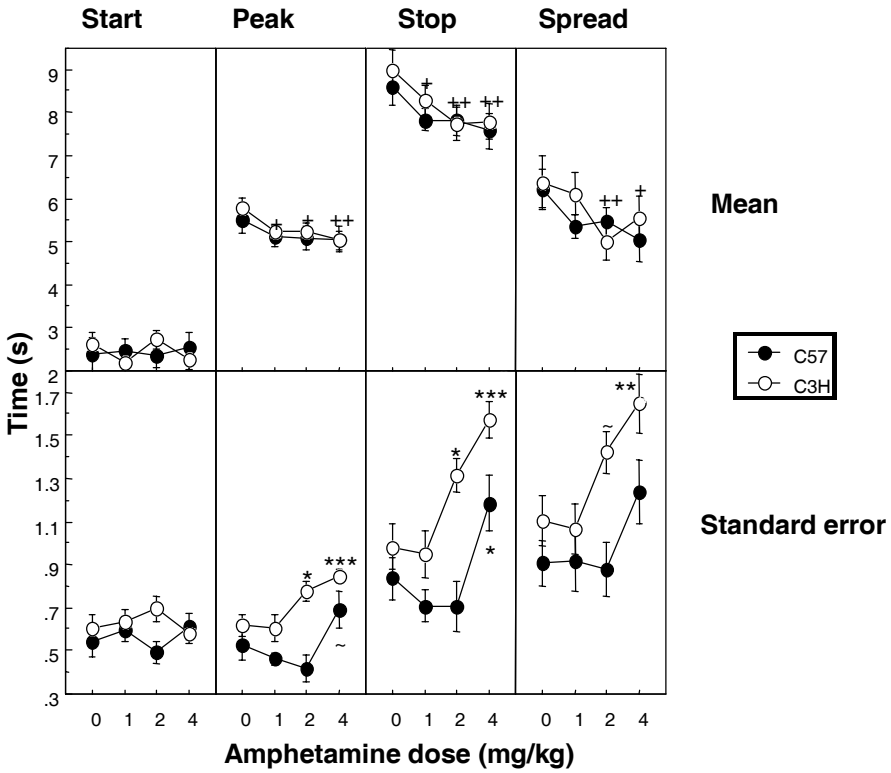

Figure 9. Mean (A) and standard error (B) of the trial-by-trial parameters for both strains treated with d-amphetamine. Pluses indicate significant differences between a dose and saline, without regard for the strain (+: $p < 0.05$, ++: $p < 0.01$). Asterisks indicate significant differences between a dose and saline, for each strain separately (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$). ~: indicates a marginally significant effect ($0.1 < p < 0.05$).

Although the variability of the start did not change with d-amphetamine, the variability of the peak, spread and stop time was significantly affected by the drug, *F*s(3, 81) > 5.49. The C3H mice showed considerably more variability in all measures: Strain Main effects, *F*s(1, 27) > 15.2, and seemed more sensitive to d-amphetamine , as variability was increased at the medium and high doses for the peak and stop scores: Planned comparisons between saline and medium dose, and against the high dose, peak, *F*s(1, 45) > 6.39. The variability for the C57 was increased for the peak measure only at the highest dose, $F(1, 36) = 5.19$. The interaction between dose and strain, however, only showed a nonsignificant trend for the peak time measure, $F(3, 81) = 2.55$.

Discussion

We have shown good temporal perception in two different strains of mice that differ greatly in their behavior, physiology, and pharmacological response. Both mouse strains are well suited for complex operant procedures such as the PI. C57 mice appeared to be faster during the acquisition of the PI procedure as they developed better inhibition of responding during the nonreinforced period after the scheduled reinforcement time.

The drop in responding after reinforcement time developed as training progressed, supporting the idea that performance in the peak procedure depends on a successful discrimination between a period, with its associated temporal cues, that ends in reinforcement and a following period in which temporal cues predict nonreinforcement (Papini & Hollingsworth, 1998). As in any nonobvious discrimination, good performance requires considerable training. If peak performance were based solely temporal generalization (i.e., the animal has a representation of the time of reinforcement and responds at the maximum expectancy), as scalar expectancy theory predicts (Gibbon et al., 1984), then it could be argued that better peak performance would be present on the first presentation of a nonreinforced trial. At least it could be expected that responding would somehow show a disruption after the usual reinforcement time, which was clearly not the case. On the other hand, if the discrimination involves only periods within a peak trial, more training should result in progressively improved performance. However, in some cases, such as in the present experiments, response curves evolve into a two-peak biphasic pattern. This happens mostly with short intertrial intervals, with the second response bump disappearing when the intertrial interval is lengthened (Kirkpatrick-Steger et al., 1996). This suggests a more complex discrimination, involving within and between trial periods. The second peak could be the result of a "harmonic" set by an underlying oscillator that establishes the temporal base of the timing apparatus. Alternatively, the second peak could reflect the expectation of the next trial, a clear possibility in schedules that include a fixed intertrial interval.

Pharmacology of Peak Performance

Scopolamine is a muscarinic receptor blocker that induces both amnesia and sedation in humans (Smith et al., 1976), but amnesia and hyperactivity in

rodents (Vives & Mora, 1986). Scopolamine disrupted performance in the C3H mice by reducing response rate and flattening the relative response curve. Physostigmine blocked the effect on the absolute response rate, but not the flattening of the relative response rate. Although physostigmine did not have an apparent effect on its own on absolute or relative responding, it significantly decreased variability as shown by the trial-by-trial analysis; these results are partially consistent with other published data (Meck & Church, 1987a) with the difference that this previous study found a leftward shift of the response function, whereas in our case the effect was mainly seen in a reduction of the variability. This discrepancy may be due to a differential sensitivity to the drugs between rats and mice or to other factors that may be uncovered by more extensive dose-response studies.

Evidence that the cholinergic system is involved in temporal information processing also comes from studies showing that cholinergic supplementation in neonates improves spatial and temporal memory, and cholinergic deficit impairs divided attention and accelerates age-related declines in temporal processing (Meck et al., 1988; Meck & Williams, 1997). Our results are also consistent with some unpublished results from our laboratory using the C57B/l6 strain in the Morris water maze in which physostigmine did not block a scopolamine-induced increase in escape latencies during training and drug treatment, but partially reversed performance deficits during a probe trial after training and drug treatment had been completed. These results suggest scopolamine has effects on different brain areas that can be differentially blocked by physostigmine.

The C3H strain has been proposed as a model for ADHD because these mice respond to low doses of d-amphetamine with hypolocomotion while other strains, including the C57, do not (Helmeste & Seeman, 1982). We expected to find differential sensitivity to the effects of methylphenidate, and indeed found that the C3H were slightly more sensitive to the highest dose of methylphenidate, which slightly but significantly shifted responding to the left as shown by both the relative response and the trial by trial analyses.

The increased sensitivity of the C3H mice to the effects of d-amphetamine and methylphenidate is probably due to the increased D2 receptor binding in the striatum (Helmeste & Seeman, 1982) or other brain loci. In our study, the disruptive effects of d-amphetamine on absolute responding and on trial-by-trial variability were significant at the medium dose in the C3H mice but only at the high dose in the C57 mice, suggesting a shift to the left of the dose response curve of the C3H mice. The effects on the position of the peak time, however, were very similar on both strains, suggesting different brain circuits may mediate these effects.

The shift of the peak time to the left induced by a low dose of d-amphetamine is consistent with previous work suggesting that the time-keeping mechanism is affected by dopaminergic agonists in a way compatible with an acceleration of the internal clock or pacemaker (Meck & Church, 1987a; Meck 1996). It could be argued, however, that a clock effect should affect the position of the peak by changing both the start and stop positions. In our case, the shift in the peak was due to a shortening of the stop, but not of the start time, which argues for a different mechanism such as a disruption of sustained attention needed to complete a trial with a good performance level. Such a failure to sustain attention

during the whole trial may mean that responding will be prematurely terminated. In other words, a deficit in sustained attention will result in prematurely and randomly truncated response bursts, which will lead to an apparent left shift in the peak time (and stop time) and, at the same time, to an increased variability of both dependent measures. This is the pattern seen at medium and high doses of d-amphetamine for the C3H mice and at the high dose for the C57 mice. An impairment of sustained attention, however, cannot explain the pattern seen at low doses of d-amphetamine, for which a shortening of the peak was accompanied by a slightly decreased variability. Moreover, scopolamine, which disrupts sustained attention in humans (Broks et al., 1988), increased variability in mice performance without affecting the peak time.

On the other hand, start and stop distributions are very differently shaped, with the start time distribution being truncated at zero and skewed to the right, and the stop distribution being more symmetrical (Brunner et al., 1997). Because of the truncation at zero, it remains a possibility that the insensitivity of the start time to pharmacological manipulation is simply due to a floor effect. Another interpretation of the effects of d-amphetamine, which is consistent with our results (Chiang et al., 2000), implies that d-amphetamine leftward shifts of timing function are not due to an increased speed of the internal clock but rather to other factors involving decision mechanisms. Chiang et al. (2000) demonstrated that the hypothesis of an accelerated speed of the clock is consistent only with data originating from temporal reproduction tasks but not with temporal discrimination tasks.

It has been argued that shifts in the peak time can be seen with an acute drug treatment (as in the present case) and that under chronic treatment the subject updates its memory of the reinforcement time as it repeatedly encodes reinforcement time with an accelerated internal clock (Meck & Church, 1987b; Meck, 1996). Although it is possible that an acceleration of the internal timing mechanism leads to a higher sensitivity to time and, therefore, better temporal information processing, our data suggest d-amphetamine over shifted the peak time. In fact, whereas the peak time with saline injection was about 30 s, the highest dose of d-amphetamine shifted the peak time to about 25 s. Therefore, although the sharpening of the response rate at low doses of d-amphetamine suggests the drug may be described as a cognitive enhancer (as it results in possibly faster processing), the trial-by-trial data shows it somehow impaired temporal performance by excessively shifting the peak toward the beginning of the trial.

The present results supports the hypothesis that time perception depends on both dopaminergic and cholinergic systems (Malapani et al., 1998; Meck & Church, 1987a). Electrophysiology and high-speed voltammetry studies using a delayed reinforcement procedure have shown that dopamine release in the nucleus accumbens is maximal at the time of the response, whereas dopamine release is inhibited at the time of the reinforcer (Kiyatkin, 1994; Schultz et al., 1997). These studies suggest that the expectancy of reward is being encoded by the temporal changes in the dopamine signal. It is therefore not surprising that a task that basically uncovers reward expectancy is very sensitive to dopaminergic manipulations. In Parkinson's patients, for example, dopaminergic dysfunction in the striatum results in a temporal information-processing deficit, which disappears

when patients are treated with levodopa plus apomorphine, and reappears when patients go off the treatment (Malapani et al., 1998).

We have extended previous results in rats and humans showing a strong control of temporal information processing by dopaminergic and cholinergic agents suggesting that there is a common neurobiological basis underlying time perception. Our data also show that mice are well tuned to the complexities of operant tasks and are also very sensitive to drug manipulations. We furthermore found support for the proposal that C3H mice are a good model for at least some aspects of ADHD. We suggest, as other do, that the use of complex tasks in mice is a necessity if the neurobiology of complex processes is to be understood.

References

Allan, L. G. (1979). The perception of time. *Perception and Psychophysics,* **26**, 340-354.

Broks, P., Preston, G. C., Traub, M., Poppleton, P., Ward, C., & Stahl, S. M. (1988). Modelling dementia: effects of scopolamine on memory and attention. *Neuropsychologia,* **26**, 685-700.

Brunner, D., Fairhurst, S., Stolovitzky, G., & Gibbon, J. (1997). Mnemonics for variability: remembering food delay. *Journal of Experimental Psychology: Animal Behavior Processes,* **23**, 68-83.

Brunner, D., & Hen, R. (1997). Insights into the neurobiology of impulsive behavior from serotonin knockout mice. In D. M. Stoff & J. J. Mann (Eds.), The neurobiology of suicide: From the bench to the clinic. *Annals of the New York Academy of Sciences,* **836**, 81-105.

Brunner, D., Kacelnik, A., & Gibbon (1996). Memory for inter-reinforcement interval variability and patch departure decisions in the starling, *Sturnus vulgaris*. *Animal Behaviour,* **51**, 1025-1045.

Catania, A. C. (1970). Reinforcement schedules and psychophysical judgements: A study of some temporal properties of behavior. In W. N. Schoenfeld (Ed.), *The theory of reinforcement schedules* (pp. 1-42). New York: Appleton-Century-Croft.

Carvalho, O. M., Silva, A. J., & Balleine, B.W. (2001). Evidence of selective learning deficits on tests of Pavlovian and instrumental conditioning in α–CaMKII^{T286A} mutant mice. *International Journal of Comparative Psychology,* **14**, 161-174.

Chiang, T. J., Al-Ruwaitea, A. S., Mobini, S., Ho, M. Y., Bradshaw, C. M., & Szabadi, E. (2000). The effect of d-amphetamine on performance on two operant timing schedules. *Psychopharmacology*, **150**, 170-184.

Church, R. M., Getty, D. J., & Lerner, N. D. (1976). Duration Discrimination by Rats. *Journal of Experimental Psychology: Animal Behavior Processes*, **2**, 303-312.

Church, R. M., Meck, W. H., & Gibbon, J. (1994). Application of scalar timing theory to individual trials. *Journal of Experimental Psychology: Animal Behavior Processes,* **20**, 135-155.

Frederick, D. L., & Allen, J. D. (1996). Effects of selective dopamine D1- and D2-agonists and antagonists on timing performance in rats. *Pharmacology Biochemistry and Behavior,* **53**, 759-764.

Gibbon, J., & Church, R. M. (1990). Representation of time. *Cognition,* **37**, 23-54.

Gibbon, J., & Church, R. M. (1992). Comparison of variance and covariance patterns in parallel and serial theories of timing. *Journal of the Experimental Analisys of Behavior,* **57**, 393-406.

Gibbon, J., Church, R. M., & Meck, W. H. (1984). Scalar timing in memory. *Annals of the New York Academy of Sciences,* **423**, 52-77.

Helmeste, D. M., & Seeman, P. (1982). Amphetamine-induced hypolocomotion in mice with more brain D2 dopamine receptors. *Psychiatry Research,* **7**, 351-359.

Kacelnik, A., Brunner, D., & Gibbon, J. (1990). Timing mechanisms in optimal foraging: some applications of scalar expectancy theory. *NATO ASI Series,* **G 20**, 61-81.

King, A., McDonald, R. & Gallistel, R. (2001). Screening for mice that remember incorrectly. *International Journal of Comparative Psychology,* **14**, 232-257.

Kirkpatrick-Steger, K., Miller, S. S., Betti, C. A., & Wasserman, E. A. (1996). Cyclic responding by pigeons on the peak timing procedure. *Journal of Experimental Psychology: Animal Behavior Processes,* **22**, 447-460.

Kiyatkin, E. A. (1994). Behavioral significance of phasic changes in mesolimbic dopamine-dependent electrochemical signal associated with heroin self-injections. *Journal of Neural Transmission - General Section,* **96**, 197-214

Koob, G., Bartfai, T., & Roberts, A. J. (2001). Molecular genetic approaches to the neuropharmacology of corticotropin-releasing factor. *International Journal of Comparative Psychology,* **14**, 90-110 .

Kuribara, H., & Tadokoro, S. (1987). Characteristics of effects of repeated scopolamine administration on ambulatory activity in mice and methamphetamine sensitivity in the scopolamine-experienced mice: Comparison among 6 strains. *Japanese Journal of Pharmacology,* **45**, 551-557.

Lejeune, H., & Wearden, J. H. (1991). The comparative psychology of fixed-interval responding: Some quantitative analyses. *Learing and Motivation,* **22**, 84-111.

Malapani, C., Rakitin, B., Levy, R., Meck, W. H., Deweer, B., Dubois, B., & Gibbon, J. (1998). Coupled temporal memories in Parkinson's disease: A dopamine-related dysfunction. *Journal of Cognitive Neuroscience,* **10**, 316-331.

Maricq, A. V., Roberts, S., & Church, R. M. (1981). Methamphetamine and time estimation. *Journal of Experimental Psychology: Animal Behavior Processes*, **7**, 18-30.

Marks, M. J., Miner, L. L., Cole-Harding, S., Burch, J. B., & Collins, A. C. (1986). A genetic analysis of nicotine effects on open field activity. *Pharmacology Biochemistry and Behavior*, **24**, 743-749.

Merck, W. (2001). Interval timing and genomics: What makes mutant mice tick? *International Journal of Comparative Psychology*, **14**, 211-231.

Meck, W. H. (1996). Neuropharmacology of timing and time perception. *Brain Research and Cognitive Brain Research,* **3**, 227-242.

Meck, W. H., & Church, R. M. (1983). A mode control model of counting and timing processes. *Journal of Experimental Psychology: Animal Behavior Processes, 9*, 320-334.

Meck, W. H., & Church, R. M. (1987a). Cholinergic modulation of the content of temporal memory. *Behavioral Neuroscience,* **101**, 457-64.

Meck, W. H., & Church, R. M. (1987b). Nutrients that modify the speed of internal clock and memory storage processes. *Behavioral Neuroscience,* **101**, 465-475.

Meck, W. H., Church, R. M., & Olton, D. S. (1984). Hippocampus, time, and memory. *Behavioral Neuroscience,* **98**, 3-22.

Meck, W. H., Smith, R. A., & Williams, C. L. (1988). Pre- and postnatal choline supplementation produces long-term facilitation of spatial memory. *Developmental Psychobiology,* **21**, 339-353.

Meck, W. H., & Williams, C. L. (1997). Simultaneous temporal processing is sensitive to prenatal choline availability in mature and aged rats. *Neuroreport,* **8**, 3045-3051.

Nestler, E. J., DiLeone, R., & Monteggia, L. M. (2001). Inducible, call-targeted mutations in mice: new tools for genetically dissecting behavior. *International Journal of Comparative Psychology*, **14**, 111-122.

Ohyama, T., Horvitz, J. C., Drew, M. R., Gibbon, J., Malapani, C., & Balsam, P. D. (2000). Conditioned and unconditioned behavioral-cognitive effects of a dopamine antagonist in rats. *Behavioral Neuroscience*, **114**, 1251-1255.

Papini, M. R., & Hollingsworth, P. R. (1998). Role of nonreinforcement in the fixed-interval performance of pigeons. *Psychonomic Bulletin and Review,* **5**, 84-90.

Paule, M. G., Meck, W. H., McMillan, D. E., McClure, G. Y., Bateson, M., Popke, E. J., Chelonis, J. J., & Hinton, S. C. (1999). The use of timing behaviors in animals and humans to detect drug and/or toxicant effects. *Neurotoxicology and Teratology,* **21**, 491-502.

Roberts, S. (1981). Isolation of an internal clock. *Journal of Experimental Psychology: Animal Behavior Processes,* **7**, 242-268.

Schultz, W., Dayan, P., & Montague, P. R. (1997) A neural substrate of prediction and reward. *Science,* **275**, 1593-1599.

Smith, D. B., Clark, R. B., Stephens, S. R., Sherman, R. L., & Hyde, M. L. (1976). Physostigmine reversal of sedation in parturients. *Anesthesia and Analgesia*, **55**, 478-480.

Treisman, M. (1963). Temporal discrimination and the indifference interval: Implications for a model of the internal clock. *Psychological Monographs,* **77**, 1-31.

Vives, F. & Mora, F. (1986). Effects of agonists and antagonists of cholinergic receptors on self- stimulation of the medial prefrontal cortex of the rat. *General Pharmacology*, **17**, 63-67.

Wearden, J. H., & McShane, B. (1988). Interval production as an analogue of the peak procedure: Evidence for similarity of human and animal timing processes. *Quarterly Journal of Experimental Psychology,* **40B**, 363-375.

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