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ORIGINAL INVESTIGATION

Single-trials analyses demonstrate that increases in clock speed contribute to the methamphetamine-induced horizontal shifts in peak-interval timing functions

Matthew S. Matell · Melissa Bateson · Warren H. Meck

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Abstract
Introduction Drugs that increase dopamine (DA) transmission have been shown to produce an overestimation of time in duration production procedures as exhibited by horizontal leftward shifts of the psychophysical functions. However, the generality of these results has been inconsistent in the literature.
Materials and methods The present report evaluates the effects of five doses of methamphetamine (MAP) (0.5–1.5 mg/kg, i/p.) on two duration production procedures, the single duration peak-interval (PI) procedure and the multi-duration tri-peak procedure in rats.
Results We replicated and extended prior results by showing a dose-dependent proportional overestimation of time that was equivalent on both procedures (i.e., subjects behaved as though they expected reinforcement to be available earlier in real time). Single-trials analyses demonstrated that the reduction in peak rate that is often observed after MAP administration is due to an increase in the proportion of trials in which responding occurred at very low rates and without temporal control. However, these low-rate trials were not the source of the leftward shift in the temporal estimates. Rather, we found that the leftward shift of the PI functions was due to proportional changes in the placement of temporally controlled high-rate responding, which is consistent with a DA-mediated alteration in clock speed.

Keywords Time perception · Internal clock · Rate-dependence · Dopamine agonist

M. S. Matell (✉)
Department of Psychology, Villanova University,
800 Lancaster Avenue,
Villanova, PA 19085, USA
e-mail: matthew.matell@villanova.edu

M. Bateson
School of Biology and Psychology, Newcastle University,
Newcastle upon Tyne, UK
e-mail: melissa.bateson@ncl.ac.uk

W. H. Meck
Department of Psychology and Neuroscience, Duke University,
572 Research Drive, GSRBII-3rd Floor-Box 91050,
Durham, NC 27708, USA
e-mail: meck@psych.duke.edu

Introduction

The ability to perceive time in the seconds-to-minutes range is central to an organism's capacity to interact with its environment in an efficient manner (Brunner et al. 1992; Gallistel 1990). Investigations into the anatomical and pharmacological mechanisms underlying timing and time perception are facilitated by performing neural manipulations in animals trained to behave in a temporally controlled manner. With this design, manipulations that alter the temporal control of their behavior may be interpreted as resulting from changes in their subjective perception of time. However, it is conceivable that changes in the timing of behaviors could also result from changes in nontemporal variables, such as motivation or baseline activity levels. The purpose of the present experiment is to evaluate whether methamphetamine (MAP)-induced alterations in behavior on temporal generalization tasks result from changes in the scaling of psychological time or from other concomitant changes.

Cognitive models of interval timing (Church and Broadbent 1991; Gibbon et al. 1984; Matell and Meck

2000, 2004; Treisman 1963) have proposed that the output of an internal clock is compared to previously stored temporal memories to guide the timing of behavior. Specifically, the interval-timing system can be broken down into three information-processing stages: clock, memory, and decision (Church et al. 1991; Gibbon et al. 1984). The clock stage encompasses a set of neural processes whose output is proposed to vary in an isochronous manner (e.g., a pacemaker-accumulator in which the pulse count in the accumulator grows as a linear function of time). The output of the clock stage is compared by decision mechanisms to memories of times of biological significance, and when the current clock output value is sufficiently close to these memories, the organism initiates behaviorally relevant, temporally controlled responding. While the specific computations involved in these timing processes differ among the various cognitive models of interval timing, it was proposed that all of the models can be understood in terms of these general information-processing stages (Church 1997).

One task that was frequently utilized to examine interval timing in a variety of animals is the peak-interval (PI) procedure (Paule et al. 1999; Rakitin et al. 1998; Roberts 1981; Schneider 1969). This procedure, and a multiple duration variant of this procedure, will be used in the present experiment to evaluate the impact that dopaminergic drugs have on temporally controlled responding, and will be described here to facilitate a discussion of previous work on the role of dopamine (DA) in timing and time perception. The PI is a variant of a discrete-trials fixed-interval (FI) schedule of reinforcement. In a FI procedure, on each trial, a stimulus (e.g., a tone) is turned on, and reinforcement is provided upon the first response (e.g., press of a response lever) made by the subject after the criterion duration has elapsed (e.g., 30 s). Responses made before the criterion duration have no consequence. Averaging the subject's responses over a number of trials results in a FI scallop (Ferster and Skinner 1957) in which response rate is low at the beginning of the trial and gradually rises to a maximum around the time of reinforcement. In the PI procedure, 50% of the trials are reinforced FI trials, and the remaining 50% of the trials are probe trials, in that no reinforcement is given and the stimulus remains on for several times the criterion duration. After the passage of the criterion duration without reinforcement, the subject's average response rate gradually returns to the baseline level. Thus, plots of the average response rate as a function of time in the trial are well described by a normal distribution that "peaks" at or near the criterion duration (see Fig. 2). The mean of this distribution, the *peak time*, is used as a measure of the accuracy of the subject's temporal perception, whereas the width of the function, the *peak spread*, serves as a measure of the variability (or confidence level) of the subjects' temporal estimates. The peak spread typically grows in direct proportion to the duration being timed (e.g., see Fig. 2b), a behavioral relation referred to as the *scalar property* (Gibbon 1977).

In contrast to the smooth rise and fall of the averaged peak functions, the topography of responding on a single trial is well described by a single-step function in which the subject responds at a low rate early in the trial, abruptly switches to a high rate of responding before the criterion duration, and then abruptly returns to a low rate of responding following the criterion duration as shown in Fig. 2e,f (see also Abner et al. 2001; Cheng and Westwood 1993; Church et al. 1994; Gallistel et al. 2004). Due to trial-by-trial variability in the placement and width of the high response rate period, averaging these step functions over many trials produces the Gaussian curve-shaped peak function. The times in the trial at which a subject makes a response rate transition were interpreted as representing the times at which the decision stage signals that the currently elapsing time has become sufficiently similar or dissimilar to previously reinforced times to initiate or terminate responding, respectively (Church et al. 1994). These rate-transition points can be determined on a trial-by-trial basis by iterative fitting of a single-step function, and the correlations and variance patterns for starts and stops allow inferences into the composition and computations of the interval-timing system. For instance, the start time and stop time are positively correlated on individual trials (Cheng and Westwood 1993; Church et al. 1994). Furthermore, in subjects timing multiple durations on a single trial, the stop times of these durations are also positively correlated (Gallistel et al. 2004). Taken together, these positive correlations strongly suggest that clock speed varies on a trial by trial basis.

The information-processing stages involved in duration discrimination may be mediated by pharmacologically and anatomically distinct mechanisms. For example, by evaluating the pattern of responding after acute and chronic administration of dopaminergic and cholinergic drugs, DA neurotransmission was implicated in the clock stage, whereas cholinergic neurotransmission was implicated in the memory stage (Buhusi and Meck 2005; Meck 1983, 1996; Meck and Church 1987a,b). Specifically, acute administration of MAP, an indirect DA agonist, was reported to lead to an immediate horizontal leftward shift in the temporal response function that is proportional in magnitude to the duration being timed. Conversely, acute administration of haloperidol, a DA antagonist, leads to an immediate horizontal rightward shift, again in a proportional manner. These proportional

effects were explained by the proposal that dopaminergic agonists increase the speed of clock stage processes, such that the perceived time (i.e., the output of the clock stage) grows more rapidly than real time (Meck 1983, 1996). For example, if clock speed doubled, subjects would initiate responding 50% earlier, irrespective of the duration being timed. In contrast, an absolute, rather than proportional, shift in temporal production, irrespective of the duration being timed, might result from drug-induced changes in bottom-up attentional factors, which could alter the latency to begin timing (Penney et al. 1996). Because of the proportionality of the dopaminergic effects and because they were found in both temporal perception tasks that require a choice response (Meck 1983, 1986) and also in temporal production tasks that allows changes in response rate to be dissociated from changes in response timing (Meck 1996), these results have provided strong support for a direct role of DA in the clock stage of interval timing.

Unfortunately, the effects of DA agents on interval-timing tasks have occasionally been inconsistent with some authors reporting results that support a clock-speed interpretation (e.g., Abner et al. 2001; Buhusi and Meck 2002; Cevik 2003; Drew et al. 2003; Eckerman et al. 1987; Frederick and Allen 1996; Liao and Cheng 2005; Maricq and Church 1983; Maricq et al. 1981; Matell et al. 2004; Meck 1983, 1986, 1996), while others finding general disruptions to temporally controlled behavior, as evidenced by a flattening of the timing function (e.g., Bayley et al. 1998; Frederick and Allen 1996; McClure et al. 2005; Odum et al. 2002; Odum and Ward 2004; Santi et al. 1995). In light of these inconsistencies, recent reports have begun calling into question the mechanism by which dopaminergic drugs impact timing and time perception. For instance, Odum et al. (2002) have recently argued that DA agonists do not alter the speed of an internal clock, but rather lead to a rate-dependent alteration in behavior (Branch 1984; Dews 1981), i.e., a drug decreases the rate of behaviors that are emitted at a high rate and increases the rate of behaviors that are emitted at a low rate. These rate-dependency effects would lead to the occasionally observed flattening of the temporal response functions (Chiang et al. 2000; Eckerman et al. 1987; Frederick and Allen 1996; McClure et al. 2005; Odum et al. 2002). In addition, in those cases in which horizontal shifts in the timing functions were observed, some investigators have argued that these effects are potentially artifactual due to "discarded data from trials with long latencies…" (Odum et al. 2002).

As the results of a number of studies examining the effects of dopaminergic drugs on interval timing were mixed, and the interpretations derived from these mixed results have called into question the impact of dopaminergic drugs on clock speed, we sought to clarify the effects of MAP in rats by testing a range of doses on two different temporal production procedures, the PI procedure and the tri-peak (TP) procedure. The use of the multiple duration TP procedure allows an assessment of within-subject, between-duration changes in timing processes without relying on different signal modalities, which were shown to differentially alter clock speed due to differences in attentional factors (Penney et al. 2000). Further, by examining the effects of MAP on the pattern of responding on single trials, rather than on the aggregate data, we will evaluate whether decreases in response rate are directly tied to the leftward shift in the temporal estimates, or whether these effects are separable. Our results demonstrate that the effects of MAP are not due to a singular change in interval-timing behavior (i.e., the temporal placement of responding is not tied to the rate of responding), but results from dissociable increases in clock speed, decreases in the probability of responding, and disruptions in the temporal control of behavior.

Materials and methods

Subjects

Twenty male Sprague–Dawley rats weighing 200–300 g (Charles-River Laboratories, Raleigh, NC, USA) and approximately 4 months of age were used. Rats were housed in pairs in a 12:12 light/dark cycle with lights on from 7:00 A.M. to 7:00 P.M. Rats were given continuous access to water and were maintained at 85% free-feeding weight by being fed a ration of Purina rat chow after each daily operant session.

Apparatus

All experimental data were obtained in ten standard operant-conditioning chambers with standard manipulanda (Coulbourn Instruments, Allentown, PA, USA). A pellet dispenser delivered 45 mg of food pellets (Noyes Precision, Formula A) to a food magazine located 10 cm above the floor on the front wall. Two 4-cm retractable response levers located 2 cm from each side wall and one 4-cm nonretractable response lever placed in between the retractable levers were horizontally placed 2.5 cm above the grid floor across the front wall. A 2.5-cm Sonalert calibrated to 93 dB was mounted in between the food cup and the center response lever. A 6-W house light was located on the ceiling and was illuminated throughout the session. Each operant-conditioning chamber was housed inside a wooden sound- and light-attenuating box, and was equipped with a 10-cm ventilation fan and an eyepiece viewer for observation. An IBM PC compatible computer

running software developed in-house was attached to an electronic interface and was used to control the experimental equipment and record the data.

Drugs

Methamphetamine hydrochloride (MAP) (Sigma/RBI, Saint Louis, MO, USA) was dissolved in 0.9% saline. Five doses of the drug (0.50, 0.75, 1.00, 1.25, and 1.50 mg/kg) were used. All doses were calculated as the salt. All injections were given intraperitoneally (i.p.) as a fixed proportion of body weight (1.0 ml/kg), and were given 15 min before the start of each experimental session. Administration of the drug or a vehicle (saline) injection was given every third day with half of the rats getting one dose, and the other half getting another dose to counterbalance any "day-related" effects of the drugs. Every dose of MAP (or vehicle) was given in a pseudorandom order for a total of three times with the only constraint being that every dose had to be given in each round before a dose could be repeated. To minimize associations between the injection procedure and administration of MAP, additional vehicle injections were given to all of the rats on all "nondrug" days. Thus, a series of injections might occur as follows: 1.0 mg/kg MAP, saline, saline, 0.5 mg/kg MAP, saline, saline, 0.0 mg/kg MAP (i.e., saline given on the same day as MAP in half of the rats), saline, saline, 1.25 mg/kg MAP, etc.

Procedures

Rats were trained on either the PI (Church et al. 1994; Meck et al. 1987) or the TP procedure (Matell et al. 2004).

Autoshaping: session 1

All rats were given 1 day of autoshaping in which the two side levers were retracted and then reinserted for 1 s before the delivery of reinforcement. In addition, all levers were primed for reinforcement on a fixed-ratio 1 schedule, until 20 responses were made on each lever, or 60 min had passed.

FI training in sequential order: sessions 2–3

For rats in the TP procedure (Matell et al. 2004), a tone commenced indicating trial onset. After a 10-s interval, the first response on the left lever was reinforced, and the tone terminated for 2 s. The tone commenced again and after a 30-s interval, the first response on the middle lever was reinforced and the tone was terminated for 2 s. Again, the tone commenced and after a 90-s interval, the first response on the right lever was reinforced and the tone was terminated. A variable 55-s intertrial interval (ITI) separated trials (range 30–80 s) in this and all subsequent procedures. Responses before the criterion durations and all responses on nonprimed levers had no consequence. For rats on the PI procedure (Church et al. 1994), trials were composed in the same manner, except that only 30-s FIs were scheduled on the middle lever and the ITI followed every reinforcement. This and all subsequent procedures lasted 2 h.

FI training in random order: sessions 4–9

For rats on the TP procedure with tone onset, one of the three criterion durations was randomly selected and the appropriate lever was primed for reinforcement. No cue was given to the rat to indicate which lever/duration would be reinforced. The ITI followed after each reinforcement. For rats on the PI procedure, the FI schedule remained the same.

TP and PI training: sessions 10–32

For rats on the TP procedure, one of the three criterion durations, or a nonreinforced probe trial (duration 270–330 s) was randomly selected with replacement and the appropriate lever primed. Again, no cue was given to the rat as to which, if any, lever/duration would be reinforced. Thus, the rats were free to respond on any lever at any time in the session, though only responses made on the appropriate lever after the criterion duration were reinforced. The ITI separated all trials. For rats on the PI procedure, either a 30-s FI trial or a nonreinforced probe trial (duration 90–110 s) was randomly selected with replacement. No cue was given to indicate which trial type was in effect. Trials were separated by the ITI.

TP or PI drug testing: sessions 33–90

The behavioral procedures used in drug testing were identical to those used in TP/PI training. All rats were given three sessions of vehicle injections, at which point the drug regimen commenced as described above.

Data analysis

Individual subject data from the three drug sessions for each dose were pooled. Responses on each lever were placed into 1-s bins and organized as a function of trial type. All statistical tests were evaluated at a criterion of $p<0.05$. Greenhouse–Geisser computed adjustments to significance were performed as necessary in the event of violations to the sphericity assumption in repeated measures ANOVAs. Data from the TP procedure were normalized by the respective criterion duration to evaluate whether

different patterns of effects occurred as a function of duration.

Mean timing functions

Average PI functions, representing mean response rate on each lever as a function of time, were constructed from all probe trials. To obtain measures of temporal accuracy and precision, the data for each dose and duration were fit using a modified Gaussian function (Buhusi et al. 2005), and the mean and standard deviation of the Gaussian function were taken as the peak time and peak spread, respectively. The modified Gaussian is simply the sum of a Gaussian curve and linear ramp, the latter of which is used to minimize the contribution of responding that occurs in expectation of trial end (Church et al. 1991). The coefficient of variation (CV) was computed to evaluate whether timing on MAP remained scalar.

At high doses of MAP, temporal control was disrupted in a small number of rats, and this analysis did not provide a good fit to the data. In these cases, we used data from the next lowest dose as the estimate. To objectively decide when this should be done, we compared the fit of the Gaussian + linear ramp function to that of a linear ramp alone using a ratio of R^2 values of these fits, and replaced those data points when this ratio fell below 0.8. One rat in the PI group failed to show reliable temporal control during the baseline period and was removed from the study. For the remaining rats, this failure of temporal control occurred on a total of 5 of the 114 data points (4%) from the 10- and 30-s PI functions. In addition, due to the small number of trials and low response rates on the 90-s lever, the majority of the rats failed to show reliable temporal control for this criterion duration even on the lowest doses of MAP. For this reason, the 90-s data were not analyzed further.

Single-trials analysis

As described in the "Introduction," previous works (Cheng and Westwood 1993; Church et al. 1994; Gibbon and Church 1990; Schneider 1969) have demonstrated that responding on a single trial of the PI is well characterized by a response rate step function. The transition times into (S1) and out (S2) of the high state, the width (S2−S1), and midpoint [(S2−S1)/2] of the high state are used as indices of temporal behavior on a single trial. To calculate these indices, the rate of responding on each trial from trial onset to twice the criterion duration was iteratively fit (until the absolute residuals were minimized) with a series of three flat lines: an initial low response rate state, a high response rate state, and a terminal low response rate state. The times at which the high response state began (S1) and ended (S2) were determined, as well as the average response rate during each of the three states.

This algorithm is sensitive to local response rates and will identify very brief, nontemporally controlled, high rate "burst" responding, which occurred frequently after MAP administration, as the critical high state region rather than a longer, but lower rate period of temporally controlled responding occurring on the same trial. To minimize the degree to which these response bursts influence the obtained temporal indices, we constrained the analysis by requiring the width of the step to be greater than or equal to one fifth of the criterion duration (high state widths are typically equal to or greater than the criterion duration (see Cheng and Westwood 1993; Church et al. 1994). In addition, trials in which fit quality was poor (typically due to the contribution of burst responding at multiple points during the trial) were not included in the single-trials analysis. This exclusion criterion was objectively determined by setting $\Omega^2<0.25$ (see Fig. 2e,f for exemplars of trials with acceptably low and high fit quality). Thus, we are restricting the analysis to only those trials that are composed of temporally controlled behavior.

Results

Mean function statistics

Timing MAP produced a dose-dependent decrease in peak time in both the PI procedure and for the 10- and 30-s durations of the TP procedure as illustrated in Fig. 1a. A repeated measures ANOVA on peak times from the PI procedure demonstrated a significant effect of dose [$F(5,40)=3.61$, $p<0.01$]. A linear regression "trend" analysis, performed to evaluate whether changes in peak time were related to changes in dose of MAP in a linear manner, demonstrated that the dose–response function is linear ($p<0.05$). There was also a statistical trend for MAP to dose-dependently increase spread [$F(5,40)=2.15$, $p=0.079$], yet there was no effect of MAP on the CV [$F(5,40)=2.59$].

Likewise, a repeated measures ANOVA on normalized peak times (obtained peak time/criterion time) from the TP procedure using dose and duration as within-subject factors demonstrated a significant effect of dose [$F(5,45)=3.93$, $p<0.05$], a statistical trend of duration [$F(1,9)=4.45$, $p=0.064$], and a nonsignificant dose × duration interaction [$F(5,45)=2.45$]. A linear regression analysis again demonstrated the dose-dependent effects, as the data showed a statistical trend toward being characterized by a linear function ($p<0.10$). Normalized spreads were greater for the 10-s duration than the 30-s duration, and increased as a function of MAP, as indicated by a significant effect of duration [$F(1,9)=22.99$, $p<0.001$], a significant effect of dose [$F(5,45)=3.59$, $p<0.01$], but no interaction [$F(5,45)=0.094$].

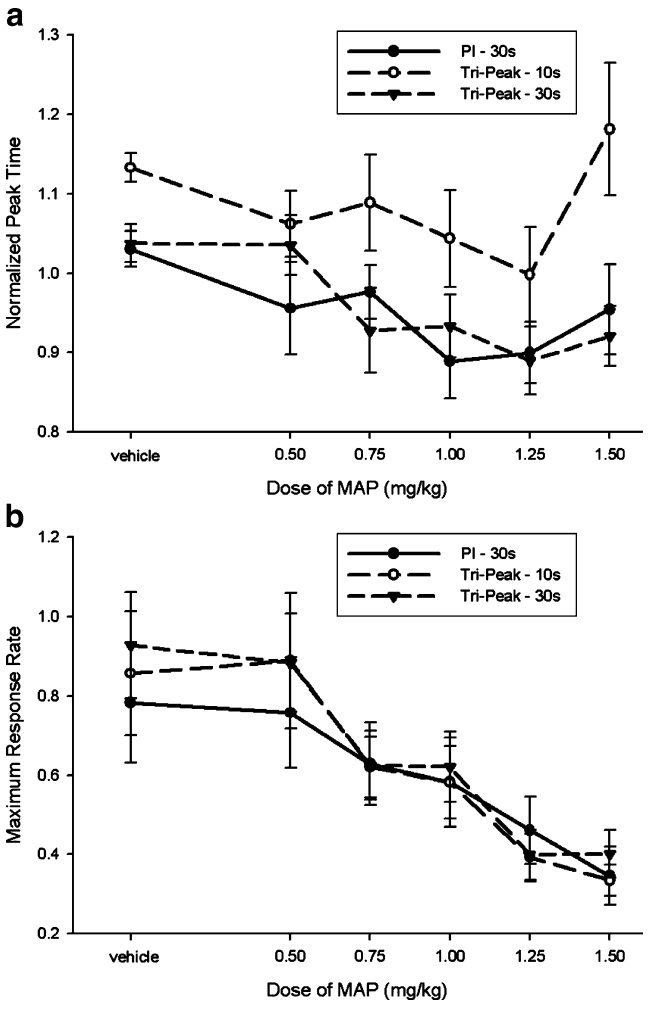

Fig. 1 **a** Normalized peak times (obtained peak time/programmed criterion time) as a function of dose of MAP. **b** Peak rate as a function of dose of MAP

Together, these effects led to nonscalar CVs that were larger for the 10-s duration than for the 30-s duration [$F(1,9)$= 7.74, $p<0.05$], and increased as a function of dose [$F(5,45)$= 4.32, $p<0.005$]. No interaction was found [$F(5,45)=0.37$]. Statistics from the fits of the mean functions are shown in Table 1.

Response rate The mean PI functions after the administration of saline and those doses of MAP that produced the maximal change in peak times (PI: 1.0 mg/kg, TP: 1.25 mg/kg) are shown in Fig. 2a,b. As illustrated in these figures, the most pronounced effect of MAP is a dose-dependent decrease in peak rate (Fig. 1b) [PI: $F(5,40)=6.098$, $p<0.05$; $F(5,45)=11.01$, $p<0.005$]. In the TP procedure, no effects of duration [$F(1,9)=0.14$] nor a duration × dose interaction [$F(5,45)=0.40$] were seen. The dose-dependent decreases in peak rate were well characterized by linear regression (PI: $p<0.05$; TP: $p<0.005$). Nevertheless, as can be seen more clearly in the normalized temporal response functions in Fig. 2c,d, there is also a leftward shift in the entire temporal distribution of responses. To ascertain whether the leftward shifts in the mean functions are directly related to the profound rate-decreasing effects of MAP, we analyzed the effects of MAP on the pattern of responding within single trials.

Individual trials

Whole trial response rates The decrease in peak rate observed in the mean function is primarily due to a substantial increase in the number of trials in which the rats responded at a low rate (i.e., a total of ≤10 responses per trial; see Fig. 3a). The transition to a greater percentage of low-response trials occurred in a dose-dependent manner [$F(5,40)=9.74$, $p<0.005$] (see Table 1 for numerical results). It is important to note that responses on the low-response trials failed to show temporal control at any dose (i.e., they were flat); and due to the low response rates, they contributed little to the shape of the mean peak functions, which was instead dominated by those trials with higher response rates (see Fig. 3b). Thus, while these low-response rate trials are primarily responsible for the decreased peak rate of the mean peak functions, they are not the basis for the horizontal shift in the peak time of the mean function as the high-response rate trials remain leftward-shifted. Furthermore, the peak response rate on these temporally controlled trials was not significantly decreased by MAP [$F(5,40)=1.168$] (see Table 1 for numerical results).

Likewise, the dose-dependent decrease in peak rate in the TP procedure is primarily due to an increase in the percentage of trials with low response rates (see Fig. 3c,e). Administration of MAP caused a dose-dependent increase in the number of trials with five or less responses on the 10-s lever [$F(5,45)=25.06$, $p<0.001$] and a dose-dependent increase in the number of trials with ten or less responses on the 30-s lever [$F(5,45)=25.06$, $p<0.001$], whereas there was no effect of duration [$F(1,9)=0.70$] nor a duration × dose interaction [$F(5,45)=1.20$] (see Table 1 for numerical results). The inflection points in the responses/trial corresponded to the transition between trials with temporal control and those with flat mean functions. Like the data from the PI, these low-response rate trials are not the basis for the MAP-induced leftward shift in the mean functions of the TP procedure. In contrast to the PI procedure, the peak rate of the temporally controlled trials decreased in a dose-dependent manner after MAP administration[$F(5,45)=2.58$, $p<0.05$], whereas there were no differences as a function of duration [$F(1,9)=0.003$] nor for the interaction [$F(5,45)=0.33$] (see Table 1 for numerical results).

Table 1 Statistics derived from the mean PI timing functions on the PI and TP procedures

Peak time		Peak spread		CV		Peak rate on high rate trials		% Low response trials		Dose (mg/kg)
PI										
30.78	0.7	13.78	0.67	0.45	0.03	1.12	0.14	25.68	5.99	Vehicle
28.22	1.76	16.40*	1.33	0.61*	0.08	1.09	0.15	26.54	4.75	0.50
28.91	1	14.99	1.22	0.52	0.05	1.03	0.1	38.10*	7.79	0.75
26.28*	1.46	18.87*	2.26	0.78*	0.16	1.02	0.12	40.29	7.42	1.00
26.63**	1.24	16.52	2.25	0.65	0.12	0.93	0.1	48.85*	7.21	1.25
27.77	1.48	20.03*	2.01	0.73**	0.07	0.87	0.1	57.72**	8.24	1.50
Tri-peak: 10 s										
11	0.18	5.87	0.35	0.54	0.03	1.04	0.15	18.31	3.91	Vehicle
10.26*	0.36	6.11	0.57	0.59	0.05	1.1	0.18	19.24	3.69	0.50
10.49	0.47	6.76*	0.42	0.65*	0.04	1	0.09	31.35	5.41	0.75
10.24	0.48	6.41	0.79	0.63	0.08	0.99	0.13	34.59*	4.97	1.00
9.55*	0.52	6.65	0.61	0.73*	0.08	0.89	0.08	54.47**	5.69	1.25
11.39	0.87	7.54	1.32	0.71	0.12	0.92	0.14	56.57**	5.98	1.50
Tri-peak: 30 s										
31.1	0.7	10.24	0.69	0.33	0.02	1.07	0.11	15.6	3.02	Vehicle
31.25	0.98	11.74*	0.75	0.38*	0.03	1.11	0.13	23.83*	4.34	0.50
27.62	1.65	14.67	2.04	0.57	0.12	0.91	0.08	37.04**	6.46	0.75
27.79**	1.3	13.38	1.52	0.52*	0.09	1.02	0.11	42.65**	7.42	1.00
25.77**	1.09	13.66*	1.43	0.55**	0.06	0.91	0.06	58.41**	7.56	1.25
27.40**	1.1	15.39*	1.33	0.57**	0.05	0.94	0.08	58.64**	6.55	1.50

The means of each statistic (peak time, peak spread, CV, peak response rate on high rate trials, and percentage of low rate trials) are given in the corresponding column for each dose of MAP (row) and in the adjacent column is the standard error.
Significant post hoc comparisons to vehicle are indicated by asterisks.
*$p<0.05$
**$p<0.01$

Step functions: timing To further identify the contributing sources of the leftward shift in the mean peak functions, we performed a single-trials analysis on the high response rate trials. The means and standard errors of the various statistics obtained from these single-trials analyses are presented in Table 2, and are described separately for each procedure below:

- *PI procedure*: MAP did not alter the start time of the high state [$F(5,40)=0.52$], but produced a dose-dependent leftward shift in the stop time of the high state [$F(5,40)=3.34$, $p<0.05$]. These statistics led to a significant decrease in the spread of the high state [$F(5,40)=7.44$, $p<0.001$], but no change in the midpoint [$F(5,40)=0.35$]. The decrease in spread as a function of dose of MAP led to a significant decrease in the CV [$F(5,40)=2.70$, $p<0.05$].
- *TP procedure*: As in the PI procedure, MAP did not alter the start times of the high states [$F(5,45)=0.69$], but produced a dose-dependent leftward shift in the stop times [$F(5,45)=5.97$, $p<0.001$]. The earlier stop times led to a dose-dependent decrease in the spread of the high states [$F(5,45)=7.37$, $p<0.001$] and a leftward shift in the midpoints [$F(5,45)=2.61$, $p<0.05$], which contributed to a decrease in the CV [$F(5,45)=3.76$, $p<0.01$]. There were significant differences in many of these statistics as a function of duration, as the high state started and ended proportionally later for the 10-s duration than the 30-s duration [start: $F(1,9)=8.38$, $p<0.05$; stop: $F(1,9)=11.24$, $p<0.01$; and midpoint: $F(1,9)=11.23$, $p<0.01$]. In contrast, duration had no impact on spread or CV, nor were there any significant interactions between dose and duration.

Step functions: response rates The rates of responding on the PI procedure during each of the three response states were unchanged by MAP administration [low state 1: $F(5,40)=0.49$; high state: $F(5,40)=1.89$; and low state 2: $F(5,40)=0.41$]. Likewise, on the TP procedure there were no significant changes in response rate resulting from MAP [low state 1: $F(5,45)=0.98$; high state: $F(5,45)=1.99$, $p=0.099$; and low state 2: $F(5,45)=0.89$]. It is important to note that the weak trend for the change in rate as a function of dose on the high state is due to the high state increasing as a function of dose [for the 10-s duration only, duration × dose interaction: $F(5,45)=2.79$, $p<0.05$]. There were significant effects of duration on the rate of the high state [$F(1,9)=23.13$, $p<0.001$] and low state 2 [$F(1,9)=70.24$,

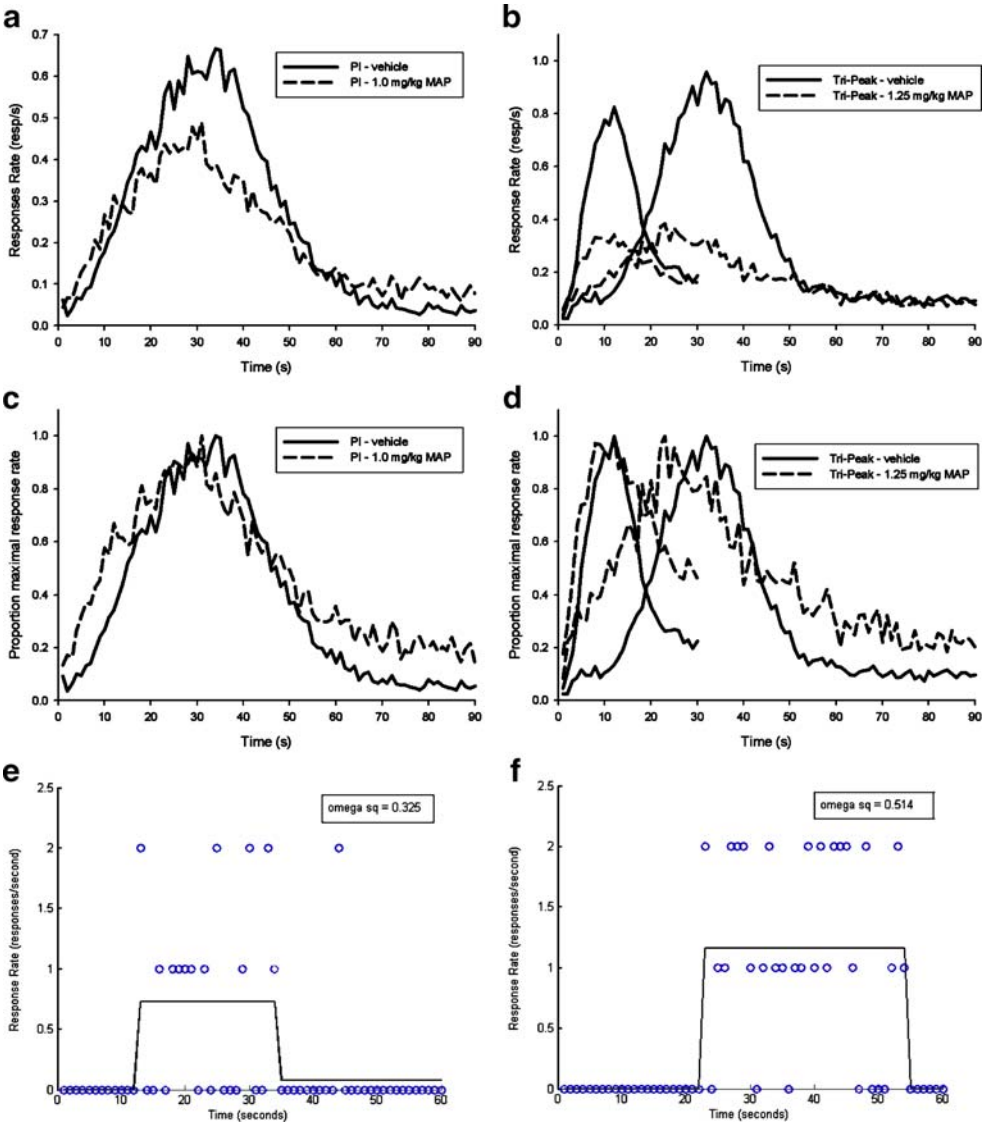

Fig. 2 PI timing functions on vehicle and MAP. Subpanel **a** (*PI*) and **b** (*tri-peak*) are raw mean functions and highlight the decrease in peak rate. In subpanel **c** (*PI*) and **d** (*tri-peak*), the functions are normalized for peak rate, and highlight the horizontal leftward shift in the entire PI timing function on MAP. The dose of the MAP plotted was that producing the largest effect in each procedure. Subpanel **e** and **f** are representative single trials response patterns from the PI procedure (1.0 mg/kg MAP) and their associated step functions with low (**e**) and high (**f**) step function fit quality

$p<0.001$], but not low state 1 [$F(1,9)=1.01$] with the response rates for the 10-s duration exceeding that for the 30-s duration in both cases. There were no dose × duration interactions for either low state. Response rates during these three states for both procedures are displayed in Table 2.

In analyzing these data, we observed individual differences in the magnitude of the changes in both the mean functions and in the statistics obtained from the single-trials analyses. We evaluated whether these differences were related to the degree of temporal control demonstrated by the rats before the onset of drug administration. To this end, we computed the correlation between the magnitude of the leftward shift (using MAP-induced changes in peak times from the mean functions or changes in the single trial statistics) and the CV of the predrug peak functions. We found no significant correlations supporting a relationship between predrug temporal control and the subsequent drug effects on either procedure.

Discussion

The current experiments tested whether the MAP-induced leftward shift in responding on temporal production procedures is mediated solely by drug-induced changes in response rate (i.e., mediated through the effects of rate dependencies), or whether these changes reflect a systematic alteration in the temporal control of behavior independent of concomitant changes in response rate. To this end, we have replicated and extended previous data (Buhusi and Meck 2002; Meck 1996) by showing that MAP produces equivalent, dose-dependent proportional leftward shifts in the mean timing functions on two temporal production procedures independent of changes in response rate. By equivalent effects, we are referring to the horizontal shifts induced by MAP, and not the relative temporal position of later responding on the 10-s compared to the 30-s levers, which existed before drug administration. Following previous interpretations regarding acute changes

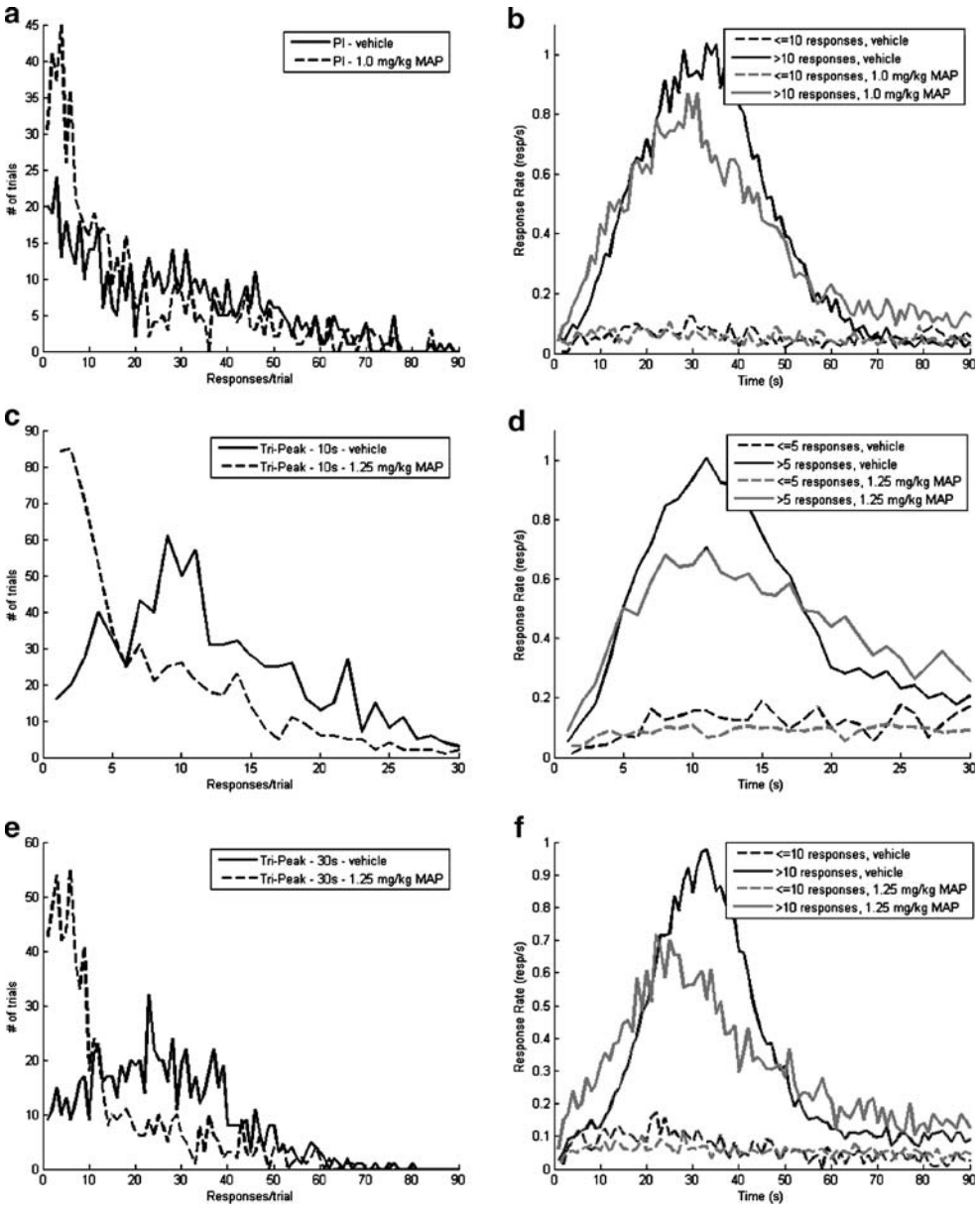

Fig. 3 **a**, **c**, **e** Histograms of the number of trials with the specified number of responses on the PI (**a**) or TP (**c**, **e**) procedures. **b**, **f** Thirty-second PI functions from those trials with greater than or less than ten responses on the PI (**b**) or TP (**f**) procedures. **d** Ten-second PI functions from those trials with greater than or less than five responses on the TP procedure

in peak times, these data are broadly consistent with a DA-mediated alteration in clock speed.

While the dose–response curves for the two procedures were significantly (PI procedure) or nearly significantly (TP, $p<0.10$) characterized by a straight line indicating that increasing the dose of MAP produces a larger leftward shift in peak time, it should be noted that the decreased leftward shift in peak times at the highest dose of MAP might be hinting at an inverted U-shaped function for temporal modulation. It remains unclear whether this inverted U-shape is indicative of a homeostatic mechanism for regulating clock speed, or whether it is due to an increase in temporally uncontrolled (uniformly distributed) burst responding biasing the peak function away from a leftward shift. Additional work utilizing microinjection techniques might allow a dissociation between burst responding and clock speed modulation, thereby allowing a cleaner dose–response evaluation of these effects.

Consistent with previous reports (Chiang et al. 2000; Eckerman et al. 1987; Frederick and Allen 1996; Odum et al. 2002), MAP dose-dependently decreased peak rate in the peak functions. To evaluate whether the obtained leftward shifts in the peak functions were due to this change in peak rate, and without making any assumptions about the underlying response patterning, we assessed the effects of MAP on the rate of responding on individual trials. As shown in Fig. 3, this decrease in peak rate was due to an increase in the proportion of trials during which the rats responded at very low rates. Furthermore, the responses made on these low-rate trials were not temporally

Table 2 Statistics derived from the single trial analyses of the PI or TP procedures

S1		S2		Spread		Midpoint		Low rate 1		High rate		Low rate 2		Dose (mg/kg)
PI														
19.52	0.74	42.59	1.12	23.07	1.14	31.05	0.76	0.09	0.01	1.14	0.11	0.12	0.03	Vehicle
19.48	1.26	41.24	1.66	21.77	1.19	30.36	1.35	0.11	0.02	1.36**	0.15	0.13	0.02	0.50
20.07	0.78	41.96	1.09	21.89	1.28	31.02	0.70	0.10	0.02	1.29	0.15	0.15	0.03	0.75
21.08	1.50	41.12	0.89	20.04*	1.34	31.10	1.03	0.10	0.02	1.34*	0.15	0.12	0.02	1.00
21.31	1.43	37.99**	1.32	16.68**	1.79	29.65	1.05	0.11	0.03	1.27	0.11	0.10	0.01	1.25
21.97	2.31	39.63	2.08	17.67**	1.32	30.80	2.10	0.09	0.02	1.18	0.13	0.12	0.04	1.50
Tri-peak: 10 s														
8.02	0.37	14.95	0.29	6.93	0.47	11.48	0.24	0.08	0.02	1.43	0.14	0.23	0.04	Vehicle
8.06	0.47	14.16*	0.21	6.10*	0.42	11.11	0.30	0.10	0.03	1.68*	0.16	0.25	0.02	0.50
8.48	0.39	14.31*	0.37	5.83*	0.38	11.40	0.33	0.09	0.02	1.70**	0.17	0.28	0.03	0.75
8.95	0.46	14.06*	0.46	5.11**	0.52	11.50	0.38	0.08	0.02	1.73*	0.17	0.24	0.04	1.00
8.93	0.60	13.88*	0.40	4.95*	0.50	11.41	0.45	0.09	0.03	1.74*	0.20	0.31	0.05	1.25
9.21	0.65	14.56	0.54	5.35*	0.39	11.89	0.57	0.09	0.02	1.65*	0.17	0.31	0.05	1.50
Tri-peak: 30 s														
22.15	0.76	41.90	1.07	19.75	1.36	32.02	0.63	0.10	0.01	1.19	0.09	0.09	0.02	Vehicle
21.80	1.19	41.32	0.92	19.51	0.79	31.56	0.99	0.12	0.02	1.18	0.17	0.10	0.02	0.50
21.77	1.43	39.62	1.34	17.86	0.93	30.70	1.31	0.10	0.02	1.10	0.16	0.08	0.02	0.75
18.81**	1.08	36.42*	1.73	17.61	1.27	27.62*	1.30	0.14	0.02	1.27	0.14	0.08	0.01	1.00
19.62	1.17	34.98**	1.43	15.36*	0.73	27.30**	1.25	0.08	0.01	1.08	0.10	0.07	0.01	1.25
20.74	1.35	36.69	1.95	15.95*	0.87	28.71	1.62	0.09	0.01	1.18	0.08	0.11	0.03	1.50

Statistics data are listed separately for each dose, procedure, and whether the analyses were constrained by the width of the obtained high state.
The standard error is provided in the column adjacent to each mean.
Significant post hoc comparisons to vehicle are indicated by asterisks.
*$p<0.05$
**$p<0.01$

controlled (i.e., plots of the response rate as a function of time were flat), thereby distinguishing these trials from high-rate trials, which demonstrated excellent temporal control. The combination of the low response rate and the uniform distribution of responding strongly suggest that this aspect of MAP's effects was not the basis for the leftward shift in the mean peak functions. Rather, the leftward shift resulted from changes that occurred on those trials in which the rats responded at relatively high rates. While we did find a small but significant decrease in aggregate peak rate as a function of dose on these high rate trials in the TP procedure, we did not find any such change in peak rate on these high rate trials in the PI procedure. Thus the current results are not consistent with the hypothesis that the leftward shift in the mean timing function is solely due to rate-dependent effects of MAP. Rather, the data are consistent with the hypothesis that MAP increases the speed of an internal clock, independent of MAP's effects on response rate. As such, these data suggest that the anatomical substrates of DA's capacity to modulate clock speed and rate may be distinct.

To further examine the effects of MAP on temporal responding, we characterized the behavior on individual trials by reference to a two-state low-high-low response rate system (Church et al. 1994; Gibbon and Church 1990) (Cheng and Westwood 1993; Gallistel et al. 2004). If MAP causes an increase in the speed of an internal clock process, then the current clock reading will progress at a faster rate, and the relative discrepancy used in the decision stage will reach both the response initiation threshold and the response termination threshold earlier in real time. Consistent with these predictions, MAP caused a dose-dependent proportional leftward shift in the stop times of all durations on both the PI and TP procedures. These MAP-induced leftward shifts in the times at which the rat stopped responding on individual trials were not accompanied by a decrease in the response rate during any of the response states. As such, these data are clear in demonstrating that the leftward shift of the mean peak functions does not result from rate-dependent effects on responding. Rather these data are consistent with DA-induced increases in the speed of a putative timing mechanism. However, in contrast to the predictions resulting from an increase in the speed of a unitary clock process, the start times were unaffected by MAP. A similar finding was also reported in mice (Abner et al. 2001), although proportional drug effects are more difficult to demonstrate for start times than for stop times due to their necessarily shorter durations. While previous behavioral work has suggested that the decisions to start and stop responding are computed independently from one another (Church et al. 1994; Gallistel et al. 2004), the current findings suggest that these independent computations may be mediated by pharmacologically separate mechanisms.

It should be noted that as constraints were made in the single-trials analyses to analyze only those trials in which the patterns of responding were of typical widths and showed temporal control, the conclusions drawn are limited to the interpretation that temporally controlled timing behavior is under the influence of a clock process that is speeded up by MAP. In contrast, we are not suggesting that these are MAP's only or even primary effects on behavior. Clearly, MAP dose-dependently decreased average response rate by increasing the number of trials in which the rats didn't respond, or responded very little. MAP also induced a disruption in the temporal control of behavior, by enhancing sporadic and brief response bursts. Both of these effects may be related to the well-known ability of drugs working at DA receptors to potentiate response "switching" (Evenden and Robbins 1983), impulsivity (Evenden and Ko 2005; Evenden and Meyerson 1999), locomotion, and stereotypical behavior (Ellinwood and Balster 1974; Fritts et al. 1997; Kuczenski and Segal 1999), which might lead the rat to abruptly abandon responding on one lever and perhaps begin responding on another lever, particularly in the TP procedure. In this respect, future work utilizing highly directed techniques (e.g., microinjection or inducible knock out/in genetic mouse models) is likely to provide greater control of the varying behavioral functions of DA drugs.

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