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SYMPOSIUM OVERVIEW

The Use of Timing Behaviors in Animals and Humans to Detect Drug and/or Toxicant Effects

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PAULE, M. G., W. H. MECK, D. E.. MCMILLAN, G. Y. H. MCCLURE, M. BATESON, E. J. POPKE, J. J. CHELONIS AND S. C. HINTON. *Symposium overview: The use of timing behaviors in animals and humans to detect drug and/or toxicant effects.* NEUROTOXICOL TERATOL **21**(5) 491–502, 1999—Behavioral paradigms applicable for use in both human and nonhuman subjects for investigating aspects of timing behavior are presented with a view towards exploring their strengths, weaknesses, and utility in a variety of experimental situations. Tri-peak, peak interval, differential reinforcement of low rate responding, and temporal response differentiation procedures are highlighted. In addition, the application of timing tasks in preclinical and clinical settings is discussed: pharmacological manipulations are providing information on the neurotransmitters involved and species differences; normative data for children are being developed; and noninvasive imaging procedures are being employed in adult human subjects to explore the involvement of specific brain areas.

Comparative psychology Time production Time estimation Temporal discrimination

AT the 1998 Annual Meeting of the Behavioral Toxicology Society, held in Research Triangle Park, North Carolina, the membership enjoyed a series of presentations that focused on the use of several behavioral procedures for studying aspects of temporal discrimination, or time perception. The following text provides synopses of the six symposium presentations along with an excellent bibliography that should serve as an important resource for all of those interested in these innovative and provocative approaches to the study of timing phenomenon in both humans and animals. Warren Meck opened the symposium with an excellent overview of the area and some important issues for thought.

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INTRODUCTION TO INTERVAL TIMING AND ITS APPLICATION TO BEHAVIORAL TOXICOLOGY

Interval timing refers to the ability of an organism to adapt its behavior to the temporal regularity of events (e.g., lights, tones, reinforcers) whose duration or spacing is in the seconds-to-hours range. Whether one advocates a Poisson pacemaker/accumulator model of interval timing with: independent stages for clock, memory, and decision processes [e.g., (21,10)]; a behavioral theory of timing in which a Poisson pacemaker initiates the transition among response states that are tied to observable patterns of behavior and organized by the delivery of reinforcement [e.g., (32,36)]; a memory-decay model based on the evaluation of trace strengths derived from external time markers (75); or a coincidence-detection model in which spiny neurons in the striatum integrate oscillatory inputs from the cortex in order to determine a unique pattern of oscillations associated with specific event durations (60), there are a number of defining hallmarks of interval timing and time perception that virtually all theories have in common and which may serve as sensitive indicators for toxicological insult. These commonalties are described below.

Prevalence Among Species

Clearly, it is advantageous for all organisms to be able to anticipate and predict the occurrence of an event in space and time. Many types of learning (e.g., Pavlovian & instrumental conditioning, as well as skilled motor learning) show sensitivity to the temporal arrangements of events in a manner suggesting that organisms are designed to be able to do the "right thing at the right time." Consequently, it is not surprising that a wide range of species (e.g., bees, fish, turtles, birds, rodents, monkeys, and human infants and adults) exhibit the ability to use temporal information in the seconds-to-hours range, and that similar psychophysical properties hold across species (70).

Scalar Property of Variance

Although there are most likely multiple sources of variability that contribute to temporal discrimination, each with their own distribution form (e.g., Gamma, Gaussian, and Poisson), the sum of these different contributions produces psychophysical timing functions that exhibit the scalar property. That is, the standard deviation of these timing functions grows proportional to the mean of the interval being timed. What this demonstrates is that the timing functions obtained for a wide variety of absolute durations will superimpose when plotted on a relative time scale. This indicates that when organisms are timing the occurrence of their responses, they scale their temporal estimates of the interval in proportion to the target time. This proportional responding is known as the "scalar property" of interval timing, and it reflects a Weber's Law-like property for the perception of time that is reflected in a wide variety of timing procedures (e.g., duration bisection, fixed-interval, peak-interval, temporal generalization, time-left, differential reinforcement of low response rates, etc.). In addition to the observation of superimposition is the constant coefficient of variation (CV). The constant CV is a mathematical correlate of the superimposition principle which states that the standard deviation of a timing function increases in proportion to its mean value and that the same CV (or Weber fraction) will obtain for the same subject across a wide range of absolute durations.

It is often useful to contrast the interval and circadian timing systems in terms of their operating characteristics and cooperation [e.g., (22,26)]. The circadian system operates with a period of about 24 hrs and is often shown to have a high level of precision but relatively little flexibility in terms of the intervals to which it can be trained and how it can be stopped and/or reset. In contrast, the interval timing system can be used to time a wide range of durations and can easily be stopped and/or reset. However, this flexibility in interval timing appears to have been bought at the cost of precision. For example, the onset of 24-hr activity-rest cycles in rodents may vary by as little as 1% of the interval, whereas the variability observed in the behavior of anticipating the delivery of food reinforcement in a 60-s peak-interval timing procedure may range from 10–40% of the interval being timed, even in well-practiced subjects. Other differences observed between these two systems suggest that they may have differential sensitivity to toxicological insults based upon whether or not the memory processes associated with the frontal cortex and hippocampus are utilized by the timing system (50,59).

Because of the ubiquity of the scalar property in interval timing, the absence of this hallmark is unusual and, thus, important to the study of neurological dysfunction (38). In particular, failure of the scalar property would indicate a malfunction of the feedback control systems designed to maintain relative time perception and ratio response rules [see (23)].

Cross-Procedural Generality

A number of behavioral procedures have been used for assessing the ability of organisms to perform temporal discriminations in the seconds-to-hours range (9). These procedures include both estimation and production procedures designed to reveal different contributions to temporal control. Well-designed procedures can be used to isolate different aspects of timing performance, including the clock, memory, and decision stages used, as well as the properties affecting response inhibition and impulsivity. Procedures such as differential reinforcement of low response rate (DRL) schedules (where subjects must space their responses according to specific time intervals) provide an extremely sensitive measure of behavioral performance under temporal control without necessarily being able to isolate the underlying timing mechanism(s). In contrast, procedures such as the peak-interval (PI) timing procedure have been developed in order to minimize the effects of response inhibition and motivational variables, while at the same time being able to isolate effects on the clock, memory, and decision stages of temporal processing (25).

With our growing confidence in the cross-procedural generality of the basic properties of interval timing, a smaller set of procedures have gained wide acceptance for use in both animals and humans. The PI procedure, in particular, has recently received increased usage (e.g., 27, 66, 68).

Precision and Accuracy

The PI procedure is a discrete-trials task that consists of a mixture of fixed-interval (FI) trials—in which the subject is reinforced for its first response after the criterion time has elapsed since the onset of the signal (e.g., light or sound)—and PI trials, in which the signal remains on for much longer than the criterion time and reinforcement is not made available. Both types of trials are followed by an inter-trial interval (ITI) during which the signal is absent. The PI procedure generates a mean response rate function that resembles a Gaussian distribution centered on the programmed time of reinforcement. The observed time of the maximum height of the PI response rate function (peak time) is taken as a measure of

timing *accuracy* and spread (e.g., the width of the response rate function at half the maximum height) is used as a measure of timing *precision.* These two measures have been used extensively in the analysis of the effects of pharmacological treatments on timing behavior [e.g., (51)]. In particular, drug and lesion-induced patterns in the horizontal placement of timing functions reveal the cause of alterations in accuracy and precision as a function of attention, clock, memory, and decision processes [e.g., (25)].

A modification of the PI procedure introduces a retention interval, called a "gap", during the presentation of the signal on unreinforced probe trials. During gap trials, a subject will typically "accumulate" how much time has passed until the signal unpredictably goes off, store that value until the signal comes on again, and then continue timing the signal and respond appropriately as though the signal had been continuous. A subject with a working memory impairment will have difficulty holding in memory the amount of time that elapsed before the gap (retention interval), and resetting of the internal clock may occur as a function of the duration of this retention interval. Typically, a subject with a hippocampal lesion will be unable to remember the part of the signal that occurs prior to a gap and will time the signal beginning after the gap as though it were a completely new trial (57).

The PI procedure may also be presented in a somewhat more elaborate version known as simultaneous temporal processing (STP) in order to assess a subject's ability to divide attention between multiple signal durations (54). Divided attention can be studied in situations where different signal modalities (e.g., auditory, tactile, and visual cues) are each paired with a unique temporal criterion (e.g., 15, 30, or 60 s) and presented concurrently in an asynchronous fashion. Frequently, these stimuli are presented in a hierarchical manner where the shorter signal durations are embedded within the longer signal durations (48,63). The assumption here is that the probability that a subject's response is determined by the signal duration—as opposed to some other factor—can vary from trial to trial. On trials in which the subject's response is not determined by signal duration, the subject is considered to be inattentive to time. Consequently, the probability of attending to signal duration, p(A), is free to vary independently for multiple signal durations, thus allowing for the calculation of the probabilities that subjects are attending to one, two, or all three of the concurrently elapsing signal durations. Under normal test conditions, subjects (e.g., rats) are apparently incapable of attending to all three signal durations concurrently on every trial. The data indicate that attention is allocated in a hierarchical manner, with the p(A) decreasing with the order of stimulus onset Ð, which is also correlated with signal duration because the shorter signal durations are embedded within the longer signal durations. Meck (48) demonstrated that the p(A) to each of three concurrently presented signal durations could be increased in a proportional manner by the administration of vasopressin. That is, attention to a particular signal increased not by an absolute amount, but by an amount proportional to the distance between the saline treatment performance and the assumed asymptotic level of performance [p(A) = 1.0]. This proportional result implies that the rate of signal processing by the subject's attentional mechanisms was increased by drug administration. Speediness of mental operations is advantageous in that more operations per unit of time can be executed without overloading the system. Thus, increasing the speed of parallel processing in an STP task produces proportional increases in the p(A) to each of the signals being attended. Additional research has indicated that the frontal cortex is involved in timing (i.e., attending to) multiple signals in parallel (e.g., 25,51,63). In this context, it is important to note that the duration of a signal is a dynamic rather than a static stimulus property. Because of the demonstrated independence of the timing processes involved in STP, it is necessary to propose that multiple accumulation processes operate in parallel and that attention be shifted among these different accumulators at a reasonably high rate in order to control the necessary response states.

Sensitivity to Perinatal Treatments

Recently, we have investigated the relationship between timing multiple stimuli simultaneously and the allocation of attentional resources as a function of perinatal choline supplementation or deficiency in adult rats (61). The variables of major interest were choline availability during prenatal development and the age of the rats at the time of behavioral evaluation. Age-related discrepancies in the content of temporal memory have been observed for aged rats trained on variants of the peak-interval timing procedure used in this study (58). These effects included an increase in peak time and a broadening of the response function as rats aged from 10 to 30 months. Our recent experiments also found an increase in peak time that interacted with age and prenatal treatment condition. Control rats demonstrated a small but significant increase in peak time as a function of age that was proportional ($11.4 \pm 0.9\%$) to the signal durations being timed. Choline deficient rats demonstrated a similar, but larger, increase in peak time as a function of age that was also proportional ($17.6 \pm 1.1\%$) to the signal durations being timed. In contrast, choline-supplemented rats did not show any reliable changes in peak time as a function of aging for either signal duration. These results are reminiscent of the finding that systemic injections of arginine vasopressin to 10–13 month old rats prevented age-related discrepancies in the content of temporal memory and the associated increases in sodium-dependent, high affinity choline uptake in the frontal cortex when they became aged [27–30 months (58)].

Although there was a significant treatment effect on peak rate, with choline-supplemented rats demonstrating the lowest response rates, peak rate did not change in any straightforward fashion as a function of aging. The changes that were observed involved an interaction among signal duration, age, and treatment such that control and choline deficient rats exhibited an age-related decline in peak rate for the 15-s signal duration, while choline-supplemented rats exhibited an increase in peak rate for the 15-s signal as a function of age. In contrast, these trends were either diminished or reversed for the 30-s signal, thereby leading to a modest but significant interaction.

The results obtained for the p(A) response measure are arguably the most interesting. Investigators have previously examined the major components of attentional processes (perceptual sensitivity, response criterion, and processing capacity) in order to determine whether they are relevant to the investigation of the neuronal basis of age-related changes in cognitive abilities (71). Our data indicated that in the STP task, rats directed their attention more to the shorter signal (15 sec) than to the longer signal (30 sec). This is most likely due to the fact that the 15-s signal indicates a shorter delay to reinforcement than the 30-s signal and, hence, is more highly valued or preferred. This difference in attentiveness to the two signals provided the opportunity to observe interactions between the different signal durations as a function of prena-

tal choline availability and age. The results indicate that for the 15-s signal, both the choline-supplemented and the choline-deficient rats allocated significantly higher levels of attention to the 15-s signal than the control rats. When examining the results for the 30-s signal, a similar pattern was observed for the choline-supplemented and control rats. The major difference here is that, in contrast to the other treatment groups, the choline-deficient rats were allocating relatively little attention to the 30-s signal. This effect in the choline-deficient rats indicates a failure of divided attention. In addition, the probability of attention to both the 15-s and 30-s signals declines reliably with age for both the control and choline-deficient rats, but not for the choline-supplemented rats. Furthermore, this age-related decline is exacerbated for the choline-deficient rats when they are timing the 30-s signal.

The proposed explanation for these changes in memory and attention is an alteration in processing speed for the brain regions that contribute to these cognitive processes. Increases in processing speed produced by prenatal choline supplementation lead to increases in the p(A) being divided among multiple signals, whereby events can be processed more efficiently and therefore allow subjects to attend to additional events. Prenatal choline supplementation is also associated with a reduction in the age-related decline of attentional processes. In contrast, prenatal choline deficiency leads to an apparent decrease in processing speed and forces rats to selectively attend to stimuli rather than process them in parallel by dividing attention among relevant events. Consequently, an increase in attention to the primary signal is observed concomitant with a large decrease in attention to the secondary signal. These effects are also associated with an acceleration of the age-related decline of attentional processes in rats that were choline deficient during gestational days 12–17 and assessed at 24–26 months of age.

Another recently developed variant of the PI procedure, called the "tri-peak" procedure, appears to be particularly well suited for the study of pharmacological and toxicological agents (40). The tri-peak procedure combines the advantages of two "classic" interval timing procedures, the PI procedure and the bisection procedure (9,46). As such, this procedure requires subjects to produce responses during a timed duration in order to track three target durations presented sequentially within a single trial. Unlike STP procedures, in which multiple durations are presented concurrently with asynchronous onsets of different signal modalities, the tri-peak procedure uses a single signal onset (and hence one modality) associated with each of the three durations. For rats, this is accomplished by pairing a different response lever with each target duration (e.g., 10, 30, and 90-s) in a "left to right" or "right to left" sequence as is commonly done in the bisection procedure. In this manner, the tri-peak procedure permits the determination of three expected times of reinforcement during a single test session using three response levers or keys. Single-session analyses can be performed on data derived from this procedure allowing for determination of correlations among "start", "stop", "middle" and "spread" response measures for each of the three durations, as well as correlations among durations in a single session (11). Additionally, one of the most important features of this procedure is the ability to test for proportionality of drug-induced changes in clock speed within a single session. Differentiating between proportional versus absolute shifts in peak time after drug administration is crucial for an analysis of the cause of the horizontal shifts in timing functions produced by dopaminergic drugs [e.g., (25,46,47,51,66)]. These improvements to the standard PI procedure allow a much greater detail of investigation of the information-processing components contributing to the behavioral and physiological variance obtained with interval-timing tasks.

In summary, it has been shown that basic interval-timing procedures [e.g., DRL, PI, & temporal response differentiation (TRD)] are highly sensitive to a variety of behavioral, pharmacological, nutritional, perinatal, and gerontological manipulations [e.g., (35,55,56,61,65)]. Consequently, it will be of interest to determine if a new generation of interval timing tasks—such as the tri-peak procedure, which allows for proportional effects on peak time to be observed within a single session, and STP procedures that require divided attention among multiple signal durations and modalities—can serve as useful tools in the study of behavioral toxicology.

Don McMillan followed with a presentation in which he highlighted recent work conducted in collaboration with Gail McClure, wherein a rat model of timing using temporal response differentiation and differential reinforcement of low response rate schedules was utilized.

SOME FACTORS DETERMINING THE EFFECTS OF DRUGS ON TIMING BEHAVIOR

Timing behaviors are usually studied using two procedures: time production or time estimation. In time estimation procedures, subjects are required to discriminate between the durations of two or more different exteroceptive stimuli. In time production procedures, the subjects are required to produce responses of predetermined durations. The purpose of this presentation is to illustrate, using time production procedures, some of the procedural factors that influence the effects of drugs and other chemicals on timing behavior. This will be attempted using temporally spaced-responding schedules (15), commonly referred to as differential reinforcement of low response rate or DRL schedules, and temporal response-differentiation schedules (44), commonly referred to as TRD schedules.

All of the experiments to be discussed were performed in adult male Sprague–Dawley rats that were food deprived to 80% of their free-feeding weights throughout all experiments. The animals were trained on the timing tasks until their performance was stable before they were exposed to drugs or chemicals.

In the first set of experiments, 6 separate groups were trained to respond under various DRL and TRD schedules (41,42). These schedules were TRD or DRL 1.0–1.3 s, TRD or DRL 4.0–5.2 s, and TRD or DRL 10–13 s (the TRD and DRL time parameters were identical). For example, under the TRD 10–13 s schedule, rats were required to hold a lever down for at least 10 s, but not more than 13 s to earn a food pellet. Under the DRL 10–13 s schedule, rats were required to space their responses at least 10 s, but not more than 13 s apart. After responding stabilized under these schedules of reinforcement, drugs were administered before the session and the percentage of responses that met the time requirements of the schedules (% correct) was determined. Relative frequency distributions of response durations (TRD schedule) or inter-response times (DRL schedules) were also plotted to determine how the patterns of timing behavior were affected.

At baseline under TRD schedules, accuracy averaged from 54 to 67% with greater accuracy displayed for the longer time durations. Under DRL schedules, accuracy ranged from 24 to 51%, again with greater accuracy displayed for the longer

time durations. Phencyclidine (PCP) produced dose-dependent decreases in accuracy under both TRD and DRL schedules, except that its effects were minimal under the DRL 1.0–1.3 s schedule. Under TRD schedules, PCP increased the frequency of short duration responses, which flattened the relative frequency distributions of response duration. The drug had similar effects on inter-response time (IRT) distributions under DRL schedules, except for animals responding under the DRL 1.0–1.3 s schedule, where the only effect of PCP was to create some long pauses in responding. With this exception, the effects of PCP on these timing behaviors did not seem to depend on the length of the response duration required, or whether the schedule was a TRD or a DRL.

Methamphetamine (MA) also produced dose-dependent decreases in accuracy under all schedules, but the effects of MA on accuracy under the DRL 1.0–1.3 s schedule were small. MA increased the relative frequency of very short response durations under all TRD schedules, and its effects were greater as the duration requirements were lengthened. Under DRL schedules, MA also increased the frequency of short IRTs, except under the DRL 1.0–1.3 s schedule, where again the only clear effect was to increase the relative frequency of long pauses in responding. Under DRL 4–5.2 and 10–13 s schedules, the distribution of IRTs with durations too short for reinforcement was much flatter than that noted for similar measures under TRD schedules. Thus, the effects of MA depended more on the schedule of reinforcement (TRD vs. DRL) than did the effects of PCP.

Under TRD schedules, Δ^9-THC had little effect on accuracy when responding was maintained under TRD 1.0–1.3 and 4–5.2 s schedules, but Δ^9-THC produced a dose dependent decrease in accuracy under the TRD 10–13 s schedule. These decreases in accuracy were caused by a large shift in the response-duration distribution toward shorter response durations. Under the DRL schedules, Δ^9-THC produced small decreases in accuracy under the DRL 4–5.2 and 10–13 s schedules. Under the DRL 4–5.2 s schedule, the IRT distribution was flattened to some extent, although the distribution of IRTs still maintained a normal distribution. There were also some increases in very long IRTs. Under the DRL 10–13 s schedule, the IRT distribution shifted to the left, although the normal shape of the distribution was retained. Thus the effects of Δ^9-THC depended on both the schedule (TRD vs. DRL) and the duration requirement of the timed response.

These experiments showed that the effects of drugs on these timing behaviors depended on the drug, the dose, the schedule procedure and the duration requirements of the timed response. The effects of PCP were similar across schedules and time parameters. The effects of MA and Δ^9-THC depended on whether responding was maintained by the TRD or DRL schedule and the time parameters required by these schedules. The DRL 1.0–1.3 s schedule was not very useful for differentiating the effects of any of these drugs.

DRL and TRD schedules are also useful for studying the behavioral toxicology of environmental chemicals. Hudzik and McMillan (30) used the TRD 1.0–1.3 s schedule to study the effects of trimethyltin (TMT) on timing behavior. Under control conditions, the animals responded within the correct time window about 50% of the time. An initial administration of 4.0 mg/kg TMT did not produce effects during the next 7 sessions, at which time a second dose was given. No effects were seen for a week, after which there was a dramatic decrease in accuracy that persisted for more than 10 days. Analysis of the relative response duration distributions showed that these effects on accuracy occurred because the distribution shifted toward shorter response durations. Eventually, these animals appeared to recover completely.

On a behavioral level, several explanations have been offered to explain the effects of drugs on timing behaviors. One possibility is that the drug disrupts internal timing mechanisms (52). An extension of this idea would be that the effects of the drug might be magnified by the decrease of reinforcer delivery when the timing behavior is disrupted. According to this notion, the drug would produce its effect on time estimation, which would result in disruption of the timed response and a decrease in percent reinforcement. The decreased reinforcement would then cause further disruption of the timed response. This does not seem to occur. Using the TRD 1.0–1.3 s schedule, McMillan et al. (45) showed that if the reinforcement window was widened on days when drug was given so that the frequency of reinforcement remained high, the effects of PCP and MA were not different than on days when the reinforcement window was not widened, and the effect of the drug was to lower reinforcement frequency. Furthermore, changing the reinforcement schedule so that only every other correct response was reinforced did not disrupt performance. In fact, timing accuracy improved. These studies suggest that short-term changes in reinforcement rate after drug administration do not contribute importantly to drug effects on the relative frequency distribution of timed responses.

It is possible that under the TRD and DRL 1.0–1.3 s schedules, time perception is not really involved. For example, under the TRD 1.0–1.3 s schedule, the conditioning of a precise motor response whose duration coincides with the reinforcement interval may have been learned, so that the procedure does not involve a time differentiation. If this is true, changing the force requirements for lever pressing might be expected to disrupt the performance by changing the proprioceptive feedback necessary to make the precise motor response. To test this hypothesis rats were trained to respond under a TRD 1.0–1.3 s schedule, and then during some sessions the force required to operate the lever was increased or decreased (43). Increasing the force requirements for lever pressing decreased accuracy, as expected, but decreasing the force requirement actually improved performance. PCP produced dose-dependent decreases in accuracy regardless of the force requirements on the lever. The decrease in accuracy was caused by an increase in the relative proportion of short response durations, and these effects were more pronounced when the lever force requirements were changed. MA also produced decreases in accuracy on this timing task, but the effects were greatly attenuated when the force requirements on the lever were decreased. These data suggest that a simple disruption of proprioceptive feedback from lever pressing is not the explanation for disruption of the timing of short-duration responses. Decreasing the force required to press the lever not only improved baseline timing performance, but also it protected against the effects of MA on response duration differentiation.

Taken together, these experiments suggest the response patterns under these time production schedules are determined by the interaction of a number of complex variables that include the drug, the dose, the schedule of reinforcement, the duration of the timed response, and the force requirements on the lever. The generation of these complex responses and the effects of drugs upon them are unlikely to yield to simple explanations, such as the proprioceptive feedback hypothesis, or the speeding up or slowing down of an internal clock. Nevertheless, these timing behaviors are very sensitive to the effects of drugs and other chemicals and may well have a place in

screening in behavioral toxicology and behavioral pharmacology (28,29).

The first two presentations centered on the use of the rat as an animal model in timing experiments. Melissa Bateson followed with a description of work conducted using an avian model.

SPECIES DEFFERENCES IN THE EFFECTS OF DOPAMINERGIC DRUGS ON CLOCK SPEED

Interval timing tasks such as the peak interval procedure have been demonstrated to provide sensitive and sophisticated means of detecting and dissociating the behavioral effects of drugs [for a review see (25)]. These tasks have been particularly useful for identifying drugs that affect clock speed versus those that produce effects on memory, motivation, attention, or the general level of motor activity.

In the peak interval (PI) procedure, the first response a subject makes after a criterion time has elapsed since the beginning of the trial will sometimes result in reinforcer delivery. In nonreinforced trials, the trial continues for two or three times the criterion time before ending, with no reinforcement. The pattern of responding in these nonreinforced, or probe, trials is used as a measure of timing ability. When the data (from well-trained subjects) from several probe trials are pooled, an approximately Gaussian distribution of responses is seen centered on the criterion time. The mean of this Gaussian function is referred to as the peak time, and reflects the accuracy with which the subject is timing. The standard deviation of the function provides a measure of the precision of timing. Precision is often described by the coefficient of variation of the function (standard deviation divided by peak time), since absolute precision is usually found to be proportional to the criterion time—a hallmark of interval timing known as the scalar property, discussed earlier. Finally, the amplitude of the Gaussian function, or peak rate, is thought to reflect the motivation of the subject and the probability that it will be reinforced. Drugs that affect clock speed are identified by first training a subject under the peak procedure in the absence of drugs. Once the subject is well trained and producing stable timing functions, test sessions are conducted in which the drug of interest is administered to the subject before the session. Drugs that are thought to increase clock speed produce a leftward shift in the peak times of the ensuing timing functions that is proportional in size to the criterion interval. Drugs that are thought to decrease clock speed produce a proportional rightward shift in the peak times of the functions. It is important to note that the effects of drugs on clock speed are identified by immediate, proportional shifts in timing functions; shifts that take time to emerge after the administration of a drug are more likely to be memory effects; and shifts that are not proportional in size to the interval being timed are better explained by effects on attention (51).

In rats, dopaminergic drugs have been found to affect clock speed. Dopamine agonists such as methamphetamine and cocaine speed up the clock, whereas dopamine antagonists such as haloperidol slow it down (46). These and other results have led to the hypothesis that clock speed can be equated with the rate of firing of dopaminergic neurons of the substantia nigra pars compacta (51). There are reasons why we should expect the fundamental aspects of the neural basis of interval timing to be common across the majority of vertebrate species. Interval timing abilities have been identified in the majority of vertebrates examined (34,70). Functionally, interval timing seems vital for survival because it is involved in both efficient foraging and perhaps all associative learning. With an ability this ubiquitous, we would predict that it evolved in a common ancestor of the present-day vertebrates, and therefore the interval timing abilities of vertebrates should share common origins and perhaps also common neural mechanisms. Given the generality of interval timing among vertebrates, it is unfortunate that there have been few attempts to identify the underlying mechanisms that might be common across species. One study of the effects of d-amphetamine on responding under the peak procedure in pigeons found leftward shifts comparable to those observed in rat studies (33), and thus goes some way towards supporting a common involvement of dopamine in clock speed in both birds and mammals. However, this study was somewhat limited in that only a single criterion time was examined, thus making it more difficult to hypothesize that the drug specifically affected clock speed rather than attentional processes. Recent studies of the effects of methamphetamine on interval timing in European starlings described below have added to our comparative understanding of the involvement of dopamine in clock speed.

Interval timing in starlings was studied using a modified version of the tri-peak procedure devised for rats. The desire was to create a version of the peak procedure in which more than one interval was timed using the same stimuli and response type for each interval. This was arranged by assigning one interval to each of three spatially differentiated response keys (e.g. left = 10 s, middle = 90 s and right = 30 s). A trial began with lights of the same color illuminating all three keys. There were three equally probable trial types. In the first, the first response to the 10-s key after 10 s had elapsed would result in reinforcement being delivered and termination of the trial. In the second, the first response to the 30-s key after 30 s had elapsed would result in reinforcer delivery and termination of the trial. In the third type, the first response to the 90-s key after 90 s had elapsed would result in reinforcer delivery and termination of the trial. No cue was given as to which of the three trial types was in operation. Thus, a well-trained subject would initially start responding on the 10-s key, and if reinforcement was not obtained after some point, it would switch to the 30-s key, and if reinforcement was not obtained there it would finally switch to the 90-s key. Trials in which the 30-second key was reinforced were accompanied by probe trials on the 10-s key, whereas probes accompanied trials in which neither the 10 nor the 30-s keys were reinforced on both the 10 and 30-s keys. An attractive and novel feature of this procedure is that not only is information acquired for more than one interval in each session, but multiple intervals are also timed within each trial. This allows one to look for changes in clock speed between trials. Once the birds were producing stable baseline timing functions on the above procedure, the effects of saline and three different doses of methamphetamine (1.5, 3.0 and 6.0 mg/kg, injected i.m. 20 minutes before the session) were investigated. Three independent effects of the drug were observed. First, methamphetamine was found to produce an immediate, proportional rightward shift in the timing functions that lasted for approximately the first third of sessions that lasted between three and four hours. This effect was unexpected and suggests that while dopamine agonists appear to affect clock speed in starlings, the effect was in the opposite direction to that observed in both rats and pigeons. The observed rightward shift was not clearly dose-dependent across the three doses investigated; however, pilot studies indicated that doses below 1.5 mg/kg did not produce any measurable effects on aspects of timing function, and doses over 6 mg/kg tended to cause severe disruption of be-

havior. Methamphetamine was also found to increase the coefficient of variation of the timing functions in the first third of the sessions with larger doses causing a greater decrease in precision. Finally, the drug also dose-dependently decreased peak rate in the first third of the session.

The effects of haloperidol on timing in starlings were also investigated using the PI procedure. Preliminary data again show that in contrast to what was found in rats, haloperidol appeared to cause a leftward shift in timing functions in the starling. This observation would suggest an increase in clock speed, although only a single criterion time has been examined to date, making it impossible to distinguish between drug effects on clock speed and attentional effects.

The general effects of these drugs on motor behavior support the paradoxical effects of dopaminergic drugs on clock speed in starlings. Analysis of video tapes of starlings injected with either methamphetamine or haloperidol showed that, in general, methamphetamine decreased activity (both flights of a particular length and appetitive pecks were reduced in frequency), whereas haloperidol caused an increase in activity (the above measures both increased in frequency). These results are in direct opposition to the observed effects of these same drugs in rats and other vertebrates. However, it was observed that, as in rats, methamphetamine caused anorexia in starlings. This finding suggests that timing and motor behaviors can be grouped together mechanistically in starlings and rats, and that the control of these types of behaviors differs between species. Eating, however, appears to be controlled by a separate mechanism(s) that functions similarly in both starlings and rats.

It is interesting to speculate why it is that dopaminergic drugs appear to have opposite effects on apparent clock speed in starlings and rats, and even in starlings and pigeons. It is not necessary for us to postulate completely different neural mechanisms to explain the observed differences. It is possible that quantitative differences in the sensitivity of dopamine receptors between pigeons and starlings could result in a qualitative difference in the effects of dopaminergic drugs on clock speed. For example, if the sensitivity of dopamine autoreceptors to both dopamine and haloperidol is greater in starlings than pigeons and rats, then it is possible that methamphetamine could result in less dopamine being released into the starling synapse because release is immediately shut down by negative feedback via autoreceptors. In a similar fashion, haloperidol administration, by blocking the negative feedback via the autoreceptors in starlings, could actually result in greater dopamine release (a similar argument has been made for why humans with attention deficit/hyperactivity disorder are paradoxically slowed down by dopamine agonists). Whatever the explanation for the difference observed between the effects of dopaminergic drugs in starlings, rats and pigeons, these results should serve as a caution to the dangers of extrapolating drug effects from one species to another, even if these species are in the same taxonomic group.

The next presentation, by E. Jon Popke in collaboration with Merle Paule, focused on the use of nonhuman primates in the study of drug effects on temporal response differentiation and provided even further comparisons of drug effects between species.

EFFECTS OF DRUGS ON TIMING BEHAVIOR IN NONHUMAN PRIMATES

Previous experiments have used temporal response differentiation (TRD) schedules to examine effects of drugs on timing behavior in rats. The results of these and other experiments using different tasks to look at aspects of timing behavior suggest that dopaminergic function is an important mediator of timing behavior in rats [see (51) for a review]. Drugs that enhance dopaminergic activity, such as amphetamine, alter timing behavior in a manner consistent with an increase in the speed of the internal "clock" (42). Drugs that reduce dopaminergic activity, such as chlorpromazine, alter timing behavior in a manner that suggests a decrease in the speed of the internal "clock" (47).

Although much research has focused on the role of dopamine in timing behavior, the dopaminergic system is not the only neurotransmitter system that has been investigated, nor is it the only system that appears to be involved. Marek and colleagues (39) reported on the effects of the serotonergic agonists fluoxetine and clorgyline to increase reinforcement rates (and to reduce response rates) in rats working under a 72-s differential reinforcement of low rate (DRL) schedule. Ferguson and Paule (14) reported that the effects of the GABAergic agonist diazepam in rats were to reduce the rate of reinforcement (without altering response rates) in rats working under a TRD 10–14 s schedule. Because neither fluoxetine, clorgyline, nor diazepam produced a clear shift in the overall distribution of responses, it is difficult to interpret these data as reflecting increases or decreases in the speed of an internal clock. Nonetheless, the results of these experiments suggest that both the serotonergic and the GABAergic systems can mediate aspects of timing behavior in rats.

Although these reports have helped to shed light on the neuropharmacology of timing behavior in rodents, little is known about the neuropharmacology of timing behavior in nonhuman primates. The purpose of this presentation is to provide data on the acute effects of drugs from a variety of pharmacologic classes on aspects of timing behavior in the rhesus monkey (*Macaca mulatta*). Amphetamine, cocaine, and chlorpromazine were used to examine dopaminergic modulation, methylenedioxymethamphetamine (MDMA) and lysergic acid (LSD) were used to assess the role of serotonergic modulation, morphine and naloxone were used to assess opioid modulation, phencyclidine (PCP) and dizocilpine (MK-801) were used to assess the role of excitatory amino acids, and pentobarbital and diazepam were used to assess the role of the GABAergic system.

Subjects in these experiments were adult males, and time production was assessed using a TRD (10–14 s) schedule as part of the National Center for Toxicological Research (NCTR) Operant Test Battery (OTB) [see (64) for details]. Under this schedule, subjects were required to depress a response lever for at least 10 but not more than 14 s. Releasing the lever within the 10–14 s window resulted in the delivery of a food reinforcer. As discussed previously, the distribution of responses in and around this 10–14 s reinforcement window is thought to provide metrics of time-based behavior. All subjects had been trained to perform the task under drug-free conditions prior to the start of the studies to be discussed.

Dopaminergic Agonists/Antagonists

Amphetamine and cocaine each reduced the rate of responding within the 10–14 s window [see (72)]. There was no indication of any rightward shift in peak responses, while at least one dose of both of these agents shifted the overall TRD response distribution to the left, hinting at a possible increase in clock speed as reported in rodents. These effects were, however, not dramatic, suggesting that in the primate, dopaminergic stimulation does not greatly affect the speed of an internal clocking mechanism. Interestingly, the dopaminergic

antagonist chlorpromazine increased the rate of responding within the 10–14 s reinforcement window, decreased the rate of responding outside the 10–14 s reinforcement window, and slightly shifted peak response durations leftward. These effects, however, occurred only at the lowest dose administered (0.01 mg/kg): higher doses decreased overall responding but did not shift the peak response times either to the right or left. These observations suggest that dopamine antagonism may improve the precision of time estimation in nonhuman primates [see (12)], and perhaps even speed up the internal clock, but not slow it down. As we have seen from earlier presentations, these results differ from those obtained from both rats (42,47) and starlings and provide further evidence that the role of the dopaminergic system in timing behavior is species-specific.

Mixed Serotonergic/Dopaminergic and Serotonergic Agonists

Methylenedioxymethamphetamine (MDMA), a mixed serotonergic/dopaminergic agonist, reduced the rate of responding within the 10–14 s reinforcement window. At an intermediate dose (3.0 mg/kg), MDMA also shifted the overall distribution of responses to the left, suggesting a possible effect of MDMA to increase in the speed of the internal clock [see (17)]. Administration of lysergic acid diethylamide (LSD), a serotonergic agonist with very little activity at dopaminergic receptors, generally reduced the rate of responding within the 10–14 s window and slightly shifted the overall response distribution to the left, albeit this effect was not dramatic nor dose-dependent [see (19)]. The effects of LSD on TRD behavior thus suggest a possible speeding up of the internal clock but do not indicate any slowing.

Opiates

Administration of morphine caused a slight rightward shift in the overall distribution of responding, but only at the lowest dose tested (0.03 mg/kg); higher doses simply suppressed responding. This observation is consistent with a decrease in the speed of the internal clock [see (73)]. Administration of naloxone, on the other hand, caused a leftward shift in the overall distribution of responding, consistent with an *increase* in the speed of the internal clock. As with morphine, the effects of naloxone were present only at an intermediate dose (0.3 mg/kg), with higher doses simply suppressing response. It is interesting to note that the effects of morphine on timing in primates differ from the reported effects of morphine on timing in rats. Meck and Church (53) reported effects of morphine in rats that suggested an increase in the speed of the internal clock. Results from the present experiments in nonhuman primates suggest a decrease in the speed of the internal clock.

Excitatory amino acids. The noncompetitive NMDA receptor antagonists phencyclidine and dizocilpine (MK-801) each shifted the overall distribution of TRD responses to the right, suggesting a reduction in the speed of the internal clock [see (7,18)]. Dizocilpine exerted these effects dose-dependently, whereas phencyclidine exerted this effect only at a low dose of 0.01 mg/kg (higher doses of phencyclidine produced a general reduction in response rates). It is important to note that these results differ from those of McClure et al. (42), who reported that phencyclidine shifted the overall distribution of responses to the left in rats, suggesting an increase in the speed of the internal clock.

GABAergics

Administration of the GABAergic agonists diazepam and pentobarbital each dose-dependently reduced the rate of TRD responding [see (13,74)]. Only the administration of pentobarbital shifted the distribution of response durations to the right, perhaps suggesting a reduction in the speed of the internal clock. The difference in the effects of diazepam and pentobarbital may be due to their somewhat different mechanisms of action. Pentobarbital and diazepam each exert their effects by increasing chloride-conductance at GABAergic channels, yet pentobarbital has an additional effect of reducing glutamate-induced depolarizations at the same concentrations. Therefore, some of the effects of pentobarbital on TRD behavior (shifting response populations to the right) may be due to its effects on the glutamatergic system and not its effects on the GABAergic system. This interpretation is consistent with the apparent effects of the glutamatergic antagonists phencyclidine and dizocilpine to shift response distributions to the right.

Thus, there appear to be clear species differences in drug effects on aspects of timing behavior, and additional research will be needed to fully understand the apparent species differences, particularly with respect to the noted effects of morphine and the NMDA receptor antagonists, PCP and dizocilpine. The issue of which animal model most closely approximates the human condition will only be determined after more comparative data are obtained.

John Chelonis, also in collaboration with Merle Paule, presented next some normative data for children performing a temporal response differentiation task. Here, not only were differences across age examined in normal children, but the performance of children identified as hyperactive was also compared to that of nonhyperactives.

SOME FACTORS THAT INFLUENCE TIMING IN CHILDREN

A variety of measures of timing have been developed for use in human and nonhuman subjects [see for example (4,24, 48,49,64,77)]. Those that have been typically used to assess aspects of timing behavior in children can be divided into time estimation, time production, time reproduction, and response inhibition procedures. Time estimation tasks require the subject to identify the length of a specific stimulus duration. For example, a 20-s tone might be presented to a subject, and he/she would be required to estimate its duration. Time production tasks require subjects to produce a specific duration using a particular response. For example, a subject might be required to hold a lever down for a specific duration of time. For time reproduction tasks, subjects are required to reproduce a time interval of a specific duration that has been previously observed. Unlike time production tasks, the subject is not told specifically what this time interval is: he/she must estimate its duration from the prior presentation of a stimulus for the interval to be reproduced. One common response inhibition procedure is the differential reinforcement of low rates (DRL) procedure. This procedure requires the subject to make a particular response at low rates, usually after a specific time interval has elapsed since the previous response.

Previous research with human subjects has demonstrated that time estimation and time reproduction become less accurate as the time interval to be estimated or reproduced increases (5,62,79,80). Performance on these tasks is also sensitive to a variety of psychological and physiological disorders such as attention deficit hyperactivity disorder (3,24), delinquency (4), schizophrenia (76), and retarded motor develop-

ment (78). Additionally, performance on both time production and time estimation tasks has been shown to improve with age (8,67).

The present research sought to extend previous findings in normal children by examining time production in a large number of subjects from 4 to 13 years of age. In addition to examining how behavior on time production tasks changed with age, the research also sought to examine the effects of gender, intelligence (IQ), and hyperactivity on time production. For this research, all children were required to produce a time interval between 10 and 14 s by holding down a response lever on an operant panel. If a child successfully produced the required interval, he/she received a nickel for that trial. Note that this is exactly the same task performed by the monkey subjects discussed previously by Jon Popke; the only difference being that, here, subjects worked for money (nickel) reinforcers instead of banana-flavored food pellets.

For the purpose of analyses in the present studies, time production intervals for each trial were divided into 4 categories. The first category included lever holds that were less than 2 s (response bursts). The second category included lever holds greater than 2 s, but less than 10 s (short duration). The third category included lever holds that were between 10 and 14 s (correct duration). The fourth category included lever holds that were greater than 14 s (long duration).

Results indicated that a larger percentage of responses made by young children and hyperactive children fell into the response burst category. Additionally, younger children tended to make more long duration responses than older children. Accurate responses significantly increased with age; however, short duration responses increased until about age 8 and then decreased thereafter. Furthermore, intelligence affected the distribution of lever hold times in children. Specifically, children with higher IQs made fewer lever holds that were less than 2 s in duration, and more lever holds in the reinforced range (10 to 14 s). Gender did not appear to influence timing ability regardless of age: boys and girls exhibited approximately equal proportions of responses for each of the four response categories. Hyperactive children actually appeared to be more precise in their timing ability, since the population of lever holds occurring within the reinforced 10–14 s window was proportionally both higher and narrower than that seen for control children.

These data illustrate that aspects of timing behavior are affected by age, intelligence, and hyperactivity, but not gender. Lever hold times of less than 2 s and lever hold times between 10 and 14 s were the most sensitive to these differences. These results suggest that the expression of timing ability is sensitive to brain development as it is correlated with age and/or IQ. Additionally, these data suggest that clinical behavioral disorders that occur in children may also manifest as differences in the performance of timing tasks.

The next speaker was Sean Hinton, who provided brain imaging data from adult human subjects performing timing tasks both in the absence and presence of psychotropic medications.

FUNCTIONAL MAGNETIC RESONANCE IMAGING AND PHARMACOLOGICAL INVESTIGATIONS OF INTERVAL TIMING IN HUMANS

The peak-interval timing procedure (peak procedure) allows behavioral assessment of the effects of pharmacological treatments on subjects timing intervals in the seconds-to-minutes range. The peak procedure and its variants offer three great strengths as tools for studying the effects of drugs on interval timing. First, analytical tools are available to associate aspects of timing performance with psychological constructs, like the internal clock, memory, and attention. Second, timing effects are separable from other performance effects, such as might be due to motivational differences. Third, memory is assessed with respect to its content (quantitatively) rather than its clarity (qualitatively). Using the peak procedure, drug manipulations can yield patterns of behavioral data from which one may infer the psychological processes that are affected. Particular patterns of responding can suggest effects on clock, memory, decision, motivation, and attentional processes that are all components of an information-processing model of interval timing (20). For example, dopaminergic (DAergic) drugs are known from animal research to influence the speed of the internal clock, which is used to perform interval-timing tasks. In rats, dopamine (DA) agonists (e.g., methamphetamine [AMPH]) cause increases in clock speed, while DA antagonists (e.g., haloperidol [HALO]) cause clock speed to slow down (46). While these behavioral findings are well established in rodent animal models, currently the only human data addressing DA's effects on interval timing were gathered from patients with Parkinson's disease (37). A primary reason for using HALO as the DAergic antagonist in interval timing studies is that this drug is known to be quite selective for DA D2 receptors (31). It is these receptors in particular that are involved in modifying the speed of the internal clock (47). Research on human interval timing to date has largely been confined to behavioral studies of normal human beings [e.g., (2,16)], although attempts have been made to study interval timing in patient populations (37,69) and using electrophysiological methods (6). In contrast, many animal studies have provided great insight into the neuroanatomy and neurochemistry of time perception (47,55,59), and a successful model of time perception has guided this research for many years (11,20). This model has lately been applied to human timing data as well (68), yet little is known either pharmacologically or anatomically about how the human brain processes event durations. Converging evidence from studies in animals and humans suggests that interval timing depends on activation of neural circuits through the frontal cortex, striatum, and thalamus that are described as frontal-striatal circuits (1). The striatum is one of a group of structures in the brain collectively called the basal ganglia, which have been thought to be involved primarily in motor functions because humans with disorders of the striatum (such as Parkinson's disease and Huntington's chorea) show pronounced motor symptoms. It has become widely appreciated in recent years, however, that the striatum may play a role in cognition as well as motor control, and time perception is one of the domains thought to be subserved by the basal ganglia. For example, rats with damage to the striatum are unable to time a previously learned duration, although their ability to make motor responses is not impaired (51). Similarly, a lesion of the frontal cortex eliminates a rat's sensitivity to DAergic drugs that would normally profoundly affect interval timing (51).

Some recent data from human research also support the role of frontal-striatal circuits in interval timing. Patients with Parkinson's disease suffer from damage to the substantia nigra, which dramatically reduces levels of the neurotransmitter dopamine in the striatum. The primary symptoms associated with the disorder are tremors, difficulty initiating movements, and muscular rigidity. A recent study found that these patients had problems with interval timing as well, and the deficit was reversed by giving apomorphine, a drug that acts directly on DA receptors in the brain (37). In patients with

Huntington's disease, the striatum deteriorates, and the most obvious symptom is uncontrolled motor activity. People with Huntington's disease also have time perception deficits beyond their motor difficulties. Taken together, the rat and human data suggest that frontal-striatal circuits may be involved in timing short intervals in the range of seconds to minutes.

The peak procedure has been combined with functional neuroimaging techniques to study the functional neuroanatomy of interval timing in normal humans (27). This study used functional magnetic resonance imaging (FMRI) to identify the brain regions activated when subjects time an 11-s signal. The data show selective activation of the frontal cortex, striatum, and thalamus, and confirm the involvement of frontal-striatal circuitry in human interval timing. Activation was also apparent in these areas when subjects timed internally without making a motor response. In addition, this circuit was not activated when subjects made a motor response uncontrolled by time.

These findings demonstrate that frontal-striatal circuitry is involved in human interval timing. However, many important questions remain about the neural mechanisms underlying this cognitive ability. For example, the scalar property refers to the instantiation of Weber's Law in the temporal domain. As with many kinds of performance, temporal discrimination shows an absolute decrease in precision as the interval being timed increases. When timing precision is scaled proportionally to the timed interval, however, relative precision across different intervals is found to be constant.

A critical question is how the scalar property, an essential feature of timing in the seconds-to-minutes range, is represented in the central nervous system. This question is part of the broader issue of how the brain times different signal durations. One possibility is that particular circuit elements of frontal-striatal circuitry may be tuned to particular intervals. If this is true, the high spatial resolution of FMRI may allow identification of anatomically distinct activations for different intervals. Another possibility is that the same neurons participate in all timing behaviors regardless of the signal duration being timed.

If identical circuits are involved in timing different intervals, then what differentiates multiple signal durations may be either the extent of activation or the rate at which neurons within frontal-striatal circuits fire. The first notion suggests that those neurons involved in interval timing may, by spreading activation, recruit additional nearby neurons to participate in the circuit. Such a model predicts an expanding region of activation as timing continues, which may be accelerated by a DAergic agonist such as AMPH to produce a faster clock speed or decelerated by the DAergic antagonist HALO to produce a slower clock speed. The second idea is that the rate of action potential generation in particular frontal-striatal circuits is itself the neural substrate of timing. This idea would be supported by localized activation that becomes more intense as timing continues, with AMPH and HALO affecting it in opposite directions. A series of experiments combining DAergic drug administration with FMRI is beginning at Duke University to try to address such questions about the neural mechanism of interval timing. By allowing direct manipulation of humans' time sense, these drug studies combined with FMRI may provide greater insight into the physiological basis of the scalar property and the neural representation of temporal information. Such inquiries will lead toward greater understanding of the mechanisms the brain uses to process time in the seconds-to-minutes range.

CONCLUSION

Several procedures are available that provide robust behavioral measures thought to provide insight into aspects of processes associated with interval timing in the seconds-to-minutes range. These can be easily automated and are readily applicable in a variety of species allowing for direct interspecies comparisons. Pharmacological and other manipulations should allow descriptions of the relative importance of specific neurotransmitters in the maintenance of timing functions and provide important information on species differences and similarities. Performance of these timing tasks by humans is associated with other important measures of brain function such as IQ. Subjects can perform these tasks repeatedly, allowing for the conduct of important longitudinal studies. While they are noninvasive, they provide important insight into the workings of the central nervous system. Coupled with powerful brain imaging techniques, it is likely that we will soon come to know which brain structures subserve the varied aspects of timing behavior knowable through the use of these procedures.

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