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Amineptine, Response Timing, and Time Discrimination in the Albino Rat

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LEJEUNE, H., I. HERMANS, E. MOCAËR, M. C. RETTORI, J. C. POIGNANT AND M. RICHELLE. *Amineptine, response timing, and time discrimination in the albino rat.* PHARMACOL BIOCHEM BEHAV **51**(2/3) 165–173, 1995.—Experiment 1 recorded the effects of single (doses of 1, 5, 10, and 20 mg/kg) and repeated intraperitoneal injections (10 mg/kg) of amineptine (a tricyclic antidepressant drug) on the performance of albino rats in differential reinforcement of low rate (DRL) of 30 s, fixed-interval (FI) of 60 s, and signalled continuous reinforcement (CRF-S^D) schedules. In the second experiment, the effects of repeated (10 mg/kg) and single injections (20 mg/kg) were assessed on the discrimination of the duration of auditory stimuli (2 and 8 s). A dose-related increase in response rates was observed in FI and DRL, correlating with a dose-related impairment in the temporal regulation of performance. However, the drug remained without effect on duration discrimination. In other respects, decreases in response latency in CRF-S^D or duration discrimination tended to indicate that the drug improved vigilance and reactivity to extraneous significant stimuli. Interpretations in terms of sensitization, tolerance, or dependency could be discarded. Our data support the hypothesis that drug effects on temporal regulation in FI and DRL are secondary to a nonspecific activation of motor activity. They question the plausibility of an antidepressant effect of the drug in humans via modulation of a timing mechanism.

Amineptine   Antidepressant   Response timing   Time discrimination   Rat

AMINEPTINE is a tricyclic antidepressant with very specific biochemical properties, mainly the inhibition of the dopamine (DA) uptake and, at higher doses, the enhancement of DA release (2,21). These dopaminergic effects are reflected by the level of homovanilic acid, a DA metabolite, in the cerebrospinal fluid (23). In the encephalon, this DA metabolite increases mainly in the limbic and striatal areas (24). Amineptine does not affect the cholinergic system and induces only a small acceleration of noradrenaline (NA) turnover (23,32).

Clinical studies emphasize its fast-acting (7 days), thymoanaleptic, and stimulating effects (11), especially a decrease of the behavioural indices of psychomotor inhibition such as apathia, apragmatism, and social withdrawal. At clinical doses, amineptine generally does not affect anxiety and insomnia (12,13,25,35,36). In rodents, symptoms classically considered to be a biochemical animal model of depression (reser-

pine-induced ptosis, hypothermia, and catalepsy) regress after amineptine injections (22). The drug also enhances spontaneous motor behaviour in mice and rats (4,30).

In other respects, animal and human data tend to support the hypothesis of a dopaminergic involvement in time measurement (8,15,16,18). It is therefore plausible to hypothesize that amineptine, with its specific dopaminergic properties, might also affect the timing mechanism. To test this hypothesis, the effect of efficient antidepressant drugs must be specifically assessed on temporal performance. Data available so far have been inconclusive; they show that antidepressants do not systematically improve the accuracy of timing behavior, as can be seen from a series of experiments undertaken with the differential reinforcement of low rate (DRL) 72-s schedule. For example, tricyclic antidepressants such as desipramine or imipramine decrease the response rate and increase the rein-

forcement rate without altering the profile of the interresponse time (IRT) distribution, whose peak is shifted to the right (17,27,28,34). The same conclusion could be reached with MAO inhibitors (14,19). However, other potential or atypical antidepressants such as buspirone and gepirone alter the IRT distribution at the same doses that increase the reinforcement rate (7,26,28). Still other antidepressants with stimulating properties, such as nomifensine or bupropion, increase the response rate and impair the temporal regulation of behavior (20,33).

For amineptine in particular, enhanced anticipation and vigilance was reported in dogs on a variant of the differential reinforcement of response duration (DRRD) schedule (1). The dogs were first trained to stay on a platform and wait until the occurrence of a tone signalling reinforcer availability. In a second stage of training, the disponibility of the reinforcer continued to be signalled in a timely manner. However, for half of the trials a supplementary but identical tone (the negative signal) was present during the waiting delay. The mastery of this schedule requires the discrimination of time elapsed until both signals are sounded. It is not a pure motor-timing procedure in which the representation of the crucial time parameter has to be derived without the help of any external time-giving cue. Furthermore, this discrete-trial procedure was not designed to reveal the effect of drugs on the response rate. Interestingly, other dopaminergic antidepressant such as nomifensine or bupropion disrupt response timing in DRL by increasing the response output (20,33).

The aim of the present experiment was to clarify the role of amineptine with regard to response timing in albino rats. The behaviour of rats trained on a DRL schedule was compared to behaviour obtained on a fixed-interval (FI) and a signalled continuous reinforcement schedule (CRF-S^D), in which the subject only has to react to a signal as it occurs. These schedules might be classified according to the importance of a cognitive representation of the critical temporal parameter: maximal in DRL, minimal in CRF-S^D, and intermediary in FI. Indeed, in the last schedule, the absence of external temporal cues is partly compensated by the synchronizing effect of the periodic reinforcer delivery.

If the drug tested in the following experiments modulates only attention or vigilance, effects might be expected for CRF-S^D (increase or decrease in the latency of the response to the stimulus), but not for DRL or FI. Inversely, if the timing mechanism is targeted by the drug, effects might be expected in DRL and FI, but not CRF-S^D. Furthermore, the conjoint use of FI and DRL allows to separate a direct effect on the timing mechanism from an indirect modulation of response timing via response rate. In the former case, temporal regulation of responses should be modified in both DRL and FI, without a significant change in response rate. In the latter, a change in the temporal regulation of responses should be recorded in DRL, where response rate and response timing interact, but not in FI, where response rates and response timing can be dissociated, as classical data have shown (29).

EXPERIMENT 1

Materials and Methods

Subjects. Sixty naive Wistar male rats bred in the laboratory were housed three or four per cage in the animal quarters (temperature 21 ± 1°C). The animals were 4 months old at the beginning of the experiments. Two weeks before the first shaping session, they were progressively food-deprived until they reached 85% of ad lib body weight. Forty minutes after the end of the daily session, each rat received a limited supplemental food ration. All subjects were kept on a 12 L : 12 D cycle (lights on from 0700–1900 h).

Apparatus. The rats worked in three identical experimental cubicles (35 × 35 × 40 cm). The walls of each cubicle were made of clear Plexiglas; the floor and the ceiling were of aluminium grid. A lever requiring a 0.2-N force protruded 3 cm out of the left wall, 5 cm above the floor. The distance between the lever and the front wall was 8 cm. Reinforcers (45 mg Noyes food pellets) were dropped with a Gerbrandt pellet dispenser in a tray, 8 cm to the right of the response lever. The cubicles were located in a sound-attenuating enclosure (80 × 80 × 120 cm). Each enclosure was illuminated (60-W frosted bulb in a recess above the door of the enclosure), heated (21 ± 1°C), and ventilated, and had an observation window. For the CRF-S^D schedule, the enclosure was equipped with a loudspeaker emitting a 4000-Hz, 60-dB tone well audible to the rat (10). Experiments were monitored with PC computers from an adjacent room.

Procedure. Data from the different groups of subjects (N = 10 each) were collected over two successive experimental phases (single and repeated drug treatment), each of them involving the DRL, FI, and CRF-S^D schedules. All rats were hand-shaped at the age of 4 months and thereafter earned 25 reinforcers on CRF before specific schedule training began. For the CRF-S^D rats, shaping and CRF training took place with the tone permanently on. Each rat performed seven sessions a week, approximately at the same hour.

DRL 30-s schedule. In this schedule, a response was reinforced when it followed the preceding response by at least 30 s. Each response, reinforced or not, resets the delay timer. The final 30-s IRT was reached progressively (one session at the delays of 5, 10, and 15 s, and two sessions at the delays of 20 and 25 s). Each DRL session was limited to 40 min, 40 reinforcers, or 250 responses, whichever came first.

FI 60-s schedule. This schedule reinforced the first lever press given after 60 s had elapsed since the preceding reinforcer. Responses emitted during the interval were without consequences. The final 60-s interval was reached progressively (two sessions at FI 10 and FI 30 s). FI sessions were limited to 40 reinforcers or 40 min.

CRF-S^D schedule. This schedule reinforced the first lever press given during a 2.5-s tone sounded after the end of a 60-s intertone interval (to be exhaustive, this schedule was chained FT60/CRF-S^D). The reinforced lever press also stopped the sound and started the next 60-s intertone interval. When no lever press was given, the sound ended automatically after 2.5 s and the reinforcement opportunity was lost. Lever presses given during the intertone interval were without consequences. Before training on the CRF-S^D schedule, the subjects were first reinforced on CRF with the tone permanently on. Thereafter, the intertone interval was introduced (1 s) and the tone duration was limited to 5 s. The temporal parameters of the schedule were further modified stepwise to reach the definitive values. From CRF-S^D on, the duration of the session was limited to 40 reinforcers or 40 min.

Drug testing. Drug testing began after 40 stabilization sessions without injections, followed by 16 (single injections) or 10 (repeated injections) sessions in which the subjects received only the vehicle (distilled water). The doses were chosen to correspond with those tested on spontaneous locomotion in mice (3) and those used in humans [150–250 mg/day (30)]. All injections (drug or vehicle) were made intraperitoneally (IP)

20 min before the beginning of the daily session. For drug testing, amineptine (amineptine sodium salt; Servier) was dissolved in distilled water in a volume of 1 ml/kg body wt. After injection the rats were returned to their home cage until the beginning of the session.

Single injections. The effects of four doses of amineptine (1, 5, 10, and 20 mg/kg) were assessed. Single drug injections were separated by six daily sessions in which animals received only the vehicle. The drug administration procedure was identical for each subject and proceeded from the smallest to the highest dose of the drug.

Repeated injections. A medium dose of amineptine (10 mg/ kg) was injected for 15 consecutive sessions. The last drug injection was followed by five control sessions with vehicle injection, to check for dependence effects after withdrawal of the drug.

Behavioural measures and data analysis. In the DRL schedule, three dependent variables were recorded: the response rate (responses per minute), the mean IRT computed over the complete IRT distribution, and the coefficient of variation of the IRT distribution (standard deviation/mean IRT), which is a measure of IRT variability and another index of sensitivity to time. Measures taken from the FI schedule were the response rate and the curvature index (6). This mathematic index is computed from the cumulative response frequencies obtained in successive segments of the interval (10 6-s segments in the present case). It yields high positive values in the case of a good temporal regulation of responses (with most responses located in the last segments of the interval) and a zero value in the absence of response timing, when responses occur at a constant rate throughout the interval (i.e., when response frequencies are equivalent in the successive segments of the interval). Negative values will be obtained in the case of aberrant response timing (when response frequencies are higher in the first than in the last segments of the interval). It also is independent of the response rate. The maximum value that can be reached by the curvature index derives from the formula $N - 1/N$, with N = the number of segments of the FI. In the present case, this maximum value was 0.90. Data recorded on the CRF-S^D schedule were the poststimulus response latency (measured to the nearest hundredth of a second) and the prestimulus response rate (i.e., response rate occurring during the intertone interval).

In the first part of the experiment (single injections), the effect of each dose of amineptine was compared to the average performance from the three last days preceding the drug (baseline). Similarly, the effect of the first injection of the vehicle (dose 0) were compared to the average performance from the 3 last days without injection. These pairwise comparisons were made with an ANOVA for repeated measures and treatment as the classification criterion.

In the second part of the experiment (repeated injections), data were averaged within blocks of five successive sessions each (one block without injection, followed by two blocks with distilled water, three blocks with amineptine, and finally, one block with distilled water injections). Data from these seven successive blocks were analyzed with an ANOVA for repeated measures and block as the classification criterion. For the overall significant effect, posthoc pairwise comparisons were computed with Student's *t*-test for related samples. More precisely, these comparisons involved blocks without injection (A) and with the first vehicle injection (B), with the second vehicle injection (C) and the first amineptine injection (D), with the last amineptine injection (E) and the last vehicle

injection (F). Furthermore, to check for baseline (vehicle) and drug effect stability, comparisons were made between blocks C and F (vehicle injections that preceded and followed the drug), as well as D and E (first and last blocks of drug injection).

Results

DRL data.

Single injection of the drug. Over the successive baseline periods, mean IRT values remained close to 22 s, coefficients of variation of the IRT distributions were close to 0.60, and average response rate was low (about three responses/min). Figure 1 presents effects of the successive doses of the drug (0, 1, 5, 10, and 20 mg/kg, abscissa) in terms of the percentage of change (in plus or minus; see ordinate) with regard to the just-preceding baseline value. The dose of 1 mg/kg tended to induce a small but nonsignificant increase in the duration of the mean IRT. However, higher doses of amineptine significantly disrupted the temporal regulation of responses. Mean IRT values decreased after doses of 5 [$F(1, 9) = 7.85$, $p < 0.03$], 10 [$F(1, 9) = 7.60$, $p < 0.03$], and 20 mg/kg [$F(1, 9) = 54.65$, $p < 0.001$]. The decrease in the duration of the mean IRT was thus more pronounced after 20 than after 5 or 10 mg/kg. The coefficients of variation of the IRT distributions (not shown in the figure) increased significantly only after the dose of 20 mg/kg [$F(1, 9) = 247.92$, $p < 0.0001$]. The bottom of Fig. 1 reveals a slight and nonsignificant decrease in the response rate after a dose of 1 mg/kg. Significant increases were nevertheless recorded after injections of 5 [$F(1, 9) = 6.39$, $p < 0.05$], 10 [$F(1, 9) = 10.14$, $p < 0.02$], and 20 mg/kg [$F(1, 9) = 16.56$, $p < 0.003$]. As was the case with temporal regulation, the effect was proportional to the dose of the drug.
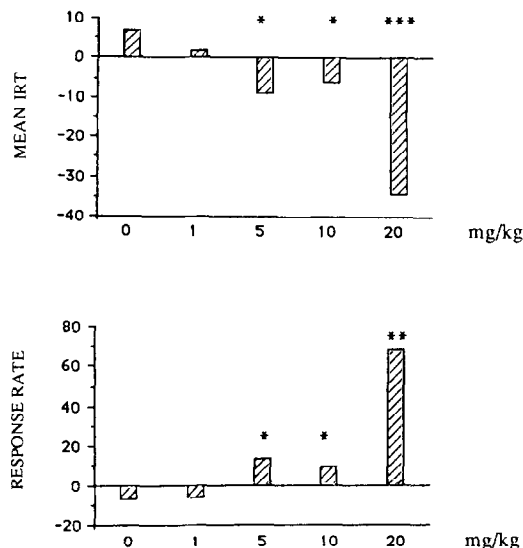

FIG. 1. Effect of single injections of different doses of amineptine (abscissa) on mean interresponse times (IRT, top) and mean response rates (bottom) in rats subjected to the DRL 30-s schedule. Data are expressed as the percent of change (+ or −; see ordinates) with regard to baseline values—i.e., the average performance from the three sessions preceding each drug injection (horizontal line at 0 on the ordinate). Ordinate scales differ according to the dependent variable under study. Significant effects: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

Repeated injections of the drug. The ANOVA computed over the seven successive blocks of sessions yielded a significant block effect on two dependent variables [mean IRT: $F(6, 54)$ = 4.33, $p < 0.002$; response rates: $F(6, 54)$ = 5.25, $p <$ 0.0005]. Drug effects on the coefficient of variation of the IRT distribution were not significant (as was the case after the single injection of 10 mg/kg). Figure 2 presents the pairwise comparisons between blocks for the mean IRT and response rates. From left to right in Fig. 2, data are expressed as the percentage of change with regard to baseline values (i.e., block A without injection and blocks C and F with vehicle injection). During these baseline blocks, absolute values of the mean IRT and response rate were similar to those from the single experiment. For the two rightmost comparisons (between blocks with drug injection, D/E, and between blocks with vehicle injection, C/F), the first block of the pair was considered the baseline. As can be seen at the top of Fig. 2, the drug significantly decreased the duration of the mean IRT [comparisons C/D: $t(9)$ = 3.899, $p < 0.005$; comparison E/ F: $t(9)$ = 4.107, $p < 0.003$], and this effect remained almost steady as long as the drug was injected (no significant difference between blocks D and E). Furthermore, the drug effect vanished as soon as injections were discontinued; no significant difference showed up between blocks C and F. Similar trends appeared for the response rate data, as can be seen at

FIG. 3. Effect of single injections of different doses of amineptine (abscissa) on the curvature index (top) and response rates (bottom) in rats subjected to the FI 60-s schedule. Other details are as in Fig. 1.
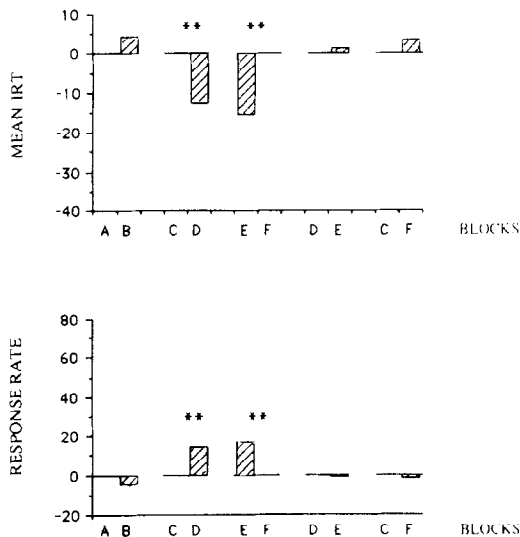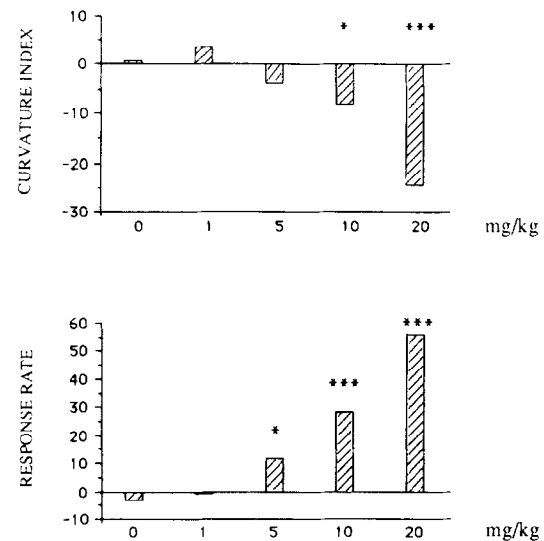

FIG. 2. Effect of repeated injections of 10 mg/kg of amineptine on mean interresponse times (IRT, top) and mean response rates (bottom) in rats subjected to the DRL 30-s schedule. Data are expressed as the percent of change (+ or −; see ordinates) with regard to baseline values. Data are averaged over successive blocks of five sessions (chronologically, from left to right on the abscissa; A: without injection; B: with vehicle injection; C: with vehicle injection; D: with drug injection; E: with drug injection; F: with vehicle injection). They also are presented pairwise (A/B; C/D; E/F; D/E; C/F). Within the three leftmost pairs, blocks without injection (A) or with vehicle injection (C and F) are considered to be baseline data with regard to the first vehicle injection (B) or drug injections (D and E). The rightmost pairs compare drug injections (D/E) and vehicle injections just preceding and following the drug (C/F). Within these pairs, the left block of data is taken as the baseline (D and C). Ordinate scales differ according to the dependent variable under study. Significant differences between pairs of blocks: *$p < 0.05$; **$p < 0.01$; ***$p <$ 0.001).

the bottom of Fig. 2. Differences between blocks C/D and E/F were significant [$t(9)$ = 4.346, $p < 0.002$; and $t(9)$ = 4.487, $p < 0.002$, respectively), whereas those between blocks D/E and C/F were not.

FI data.

Single injection of the drug. Over the baseline periods before drug injection, the average curvature index remained close to 0.65, and average response rate was about 16/min. The top of Fig. 3 shows that amineptine at 1 mg/kg tended to induce a small and nonsignificant increase of the curvature index. However, the value of this index decreased significantly after doses of 10 [$F(1, 9)$ = 8.28, $p < 0.02$] and 20 mg/kg [$F(1, 9)$ = 31.32, $p < 0.0003$]. This impaired temporal regulation was associated with a significant increase in response rates after the doses of 5 [$F(1, 9)$ = 10.53, $p < 0.02$], 10 [$F(1, 9)$ = 42.92, $p < 0.001$], and 20 mg/kg [$F(1, 9)$ = 32.12, $p <$ 0.0003], as can be seen at the bottom of Fig. 3. As was the case in DRL, effects were proportional to the dose of the drug.

Repeated injection of the drug. Two rats were accidentally lost during the experiment, which reduces the sample to eight subjects. Over blocks with vehicle injection (B, C, and F), average values of the curvature index and response rate were about 0.60 and 13, respectively.

The ANOVA revealed a significant block effect on both dependent variables [curvature index: $F(6, 42)$ = 3.02, $p <$ 0.02; response rate: $F(6, 42)$ = 11.99, $p < 0.0001$]. Figure 4 describes between-block comparisons: The top confirms that the drug significantly decreased the value of the curvature index [comparison C/D: $t(7)$ = 3.275, $p < 0.02$; comparison E/F was close to significance: $t(7)$ = 2.132, $p < 0.07$], and that this effect remained steady as long as the drug was injected (no significant difference between blocks D and E, despite a trend toward an improvement of performance over the last block of drug injection). Similarly, control blocks C and F did not significantly differ.

The bottom panel of Figure 4 presents the response rates. The drug significantly increased response rates [comparison C/D: $t(7)$ = 3.655, $p < 0.01$; comparison E/F: $t(7)$ = 6.855,

$p < 0.0003$]. No significant difference could be found between the first and the last block of drug injection (D/E), which confirms the stability of the drug effect, as well as between control blocks C and F, which indicates that this dose of the drug had no long-lasting after-effects on performance. In other respects, the first distilled water injection (B) was associated with a significant increase in response rates [comparison A/B: $t(7) = 3.389, p < 0.02$]. However, this increase was limited to block B. Indeed, differences between block B and the other blocks with vehicle injection (C and F) were not significant.

CRF-S^D data.

Single injection of the drug. Over the successive baseline periods, the average response latency was about 0.90 s, and the response rate remained close to 1.8/min. The effects of the successive doses of the drug (0, 1, 5, 10, and 20 mg/kg) can be seen in Fig. 5, with response latency data at the top. Despite the shortening of mean response latency after 20 mg/kg, no significant drug effect could be found. The bottom of the figure depicts a significant increase in presignal response rates after the highest dose of the drug [$F(1, 9) = 7.61, p < 0.03$]. The trends toward an increase in response rates seen at 5 and 10 mg/kg were not significant [$F(1, 9) = 4.62, p < 0.06$ and $F(1, 9) = 3.87, p < 0.08$, respectively].

Repeated injections of the drug. During baseline blocks C and F, average response latencies were close to 1.0 s and response rates were about two/min. The effect of the block factor was significant only on response latency [$F(6, 54) = 6.59, p < 0.0001$]. Nevertheless, the top of Fig. 6 shows that this effect did not depend on the drug, but rather on a latency decrease resulting from supplemental training. Indeed, pairwise comparisons yielded a significant difference only between blocks A and B [$t(9) = 3.829, p < 0.005$] — that is, between the last block without injection (A) and the first block with injection of the vehicle. The bottom panel of Fig. 6 depicts a trend toward an increase in presignal response rates. however, the ANOVA computed over the seven successive blocks of data was not significant [$F(6, 54) = 2.02, p < 0.08$]. This stems in particular from a single subject whose response rates far
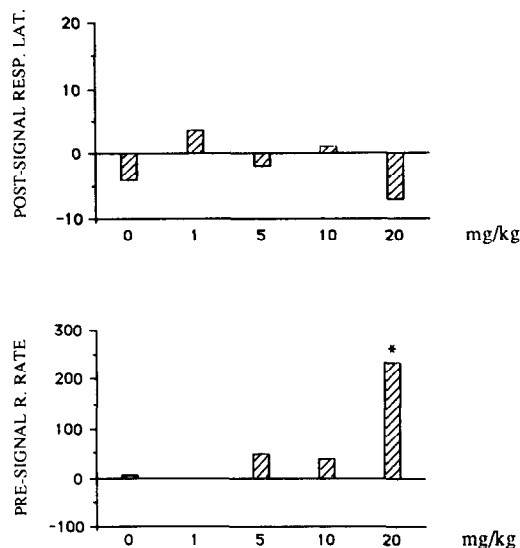

FIG. 5. Effect of single injections of different doses of amineptine (abscissa) on the postsignal response latency (top) and the presignal response rate (bottom) in rats subjected to the CRF-S^D schedule. Other details are as in Fig. 1.

exceeded those of the other rats (on average about 22 vs. three responses/min, respectively). Indeed, the ANOVA computed after discarding the data from this atypical subject yielded a just-significant block effect [$F(6, 48) = 2.54, p < 0.05$], mainly due to an increase of response rates during the first block of drug injection [comparison C/D: $t(8) = 2.931, p < 0.02$; the comparison E/F was not significant: $t(8) = 2.071, p < 0.08$]. This more local effect was further confirmed by a significant difference between blocks D and E [$t(8) = 3.12, p < 0.02$].
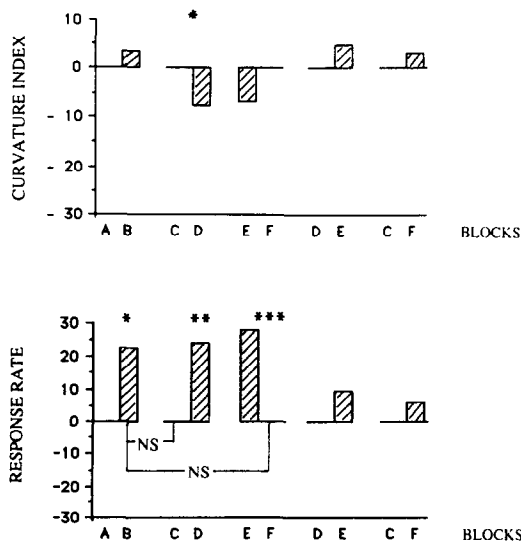

FIG. 4. Effect of repeated injection of 10 mg/kg of amineptine on the curvature index (top) and response rates (bottom) in rats subjected to the FI 60-s schedule. Other details are as in Fig. 2.
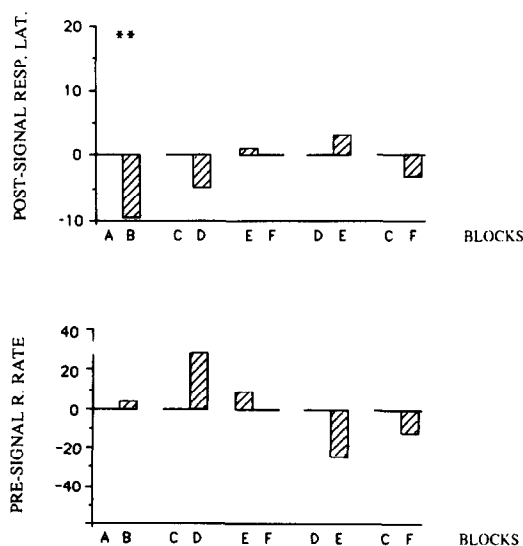

FIG. 6. Effect of repeated injection of 10 mg/kg of amineptine on the postsignal response latency (top) and the presignal response rate (bottom) in rats subjected to the CRF-S^D schedule. Other details are as in Fig. 2.

Discussion

Single injections of amineptine had two effects in FI and DRL: a decrease in the temporal accuracy of behaviour and an increase in the response rate. The magnitude of these effects was dose dependent. In CRF-S^D, the same doses remained without effect on postsignal response latency (despite a clear trend toward a decrease after 20 mg/kg). However, a significant increase in presignal response rate was obtained after the highest dose of the drug. Repeated injections of a dose of 10 mg/kg confirmed the preceding results. Significant decreases of the mean IRT (DRL) and the curvature index (FI) were associated with significant increases in the response rate. In CRF-S^D, the only significant drug effect concerned the response rate. Significant effects appeared from the very first injection of the drug (as was seen with single injections) and usually remained stable. Following the last drug injection, return to baseline values was immediate, without rebound effects. This pattern of repeated drug effects does not allow interpretations in terms of tolerance, sensitization, or dependence.

The effects of drug on the response rate were less massive in DRL than in FI, where such fluctuations had no consequences on the obtained rate of reinforcement. As such, this difference could be correlated with the degree of temporal constraint typical of each reinforcement schedule. Where response rates did not matter (FI), inhibitory control on responding could be loosened by the drug. Where they did (DRL), drug effects were partially counteracted by the response timing mechanism.

As far as temporal regulation was concerned, data did not allow us clearly to separate direct and indirect drug effects on response timing. In the former case, the temporal regulation of responses should have been modified in DRL and FI, but not the response rate. In the latter, response rate changes were expected in DRL and FI, with an alteration of response timing limited to DRL, which was not the case. Indeed, in FI, the value of the curvature index decreased significantly after injections of a dose of 10 or 20 mg/kg. The expected dissociation between response rate and response timing in FI was nevertheless partly confirmed: Effects on the response rate appeared from the dose of 5 mg/kg and were much more massive than those on response timing, especially after 10 mg/kg ($p < 0.0003$ vs. $p < 0.02$, respectively). The ANOVA computed over repeated injection data yielded the same result ($p < 0.0003$ vs. $p < 0.02$). This set of data points toward an indirect drug effect on timing via response rate, without ruling out a direct effect on the timing mechanism proper.

We needed another test to decide between these alternatives. A second experiment was undertaken using a temporal discrimination task in which timing capacities were expressed independently of response timing. Rats were trained to discriminate between two durations of an auditory stimulus (2 and 8 s). Each duration was associated with the position of a response lever on the intelligence panel. Thus, in this procedure, reinforcement depended exclusively on a choice between response opportunities and not on response location in time. Response latency data (duration between the end of a stimulus and the lever press) were also recorded to evaluate the attentional capacities of the subjects, their level of vigilance, and the decision process that precedes the emission of the response.

The following hypotheses were made: first, amineptine at 10 or 20 mg/kg will not impair the accuracy of temporal discrimination (the proportion of correct choices will not decrease); second, the drug might increase vigilance and reduce response latencies after the end of a stimulus.

Material and Methods

Subjects. We used 10 adult male Wistar rats bred in the laboratory colony. Maintenance and feeding conditions were similar to those from the preceding experiment.

Apparatus. The rats worked in a cubicle identical to those used precedingly, except that two retractable Gerbrands levers (separated by 20 cm) were located on the left wall, with the food tray in between. Furthermore, a loudspeaker was set 70 cm above the food tray. Other conditions were identical to those of Experiment 1.

Procedure. Stimulus duration discrimination was learned after training the animals to respond to the left and right retractable levers. After hand shaping, the rats were first subjected to four sessions on a CRF schedule (limited to 30 reinforcers each) with only one response lever (left or right) protruding permanently in the cubicle (sequence left-right-left-right). In a second step, the subjects were familiarized with the retraction of the lever. Four sessions were again conducted with only one lever present. During the two first sessions, the left lever protruded in the cage until 30 reinforcers had been obtained. Thereafter, it was retracted for 10 s before being presented again. Further retractions occurred after the rat earned 15, 10, and, finally, five reinforcers. The two next sessions were identical, except that the right lever was used. During sessions 9 (left lever) and 10 (right lever), the lever was retracted after each response and sessions were limited to 40 reinforcers. Finally, during sessions 11 and 12, the left or right lever was presented in random order until 20 reinforcers were earned on each.

Duration discrimination training followed. For half of the subjects, a press on the left lever was reinforced after the short stimulus duration (2 s), and on the right lever after the long stimulus duration (8 s). The opposite condition was scheduled for the remaining rats. Errorless training was conducted for five sessions (limited to 40 trials each), which started with both levers retracted out of reach. On each trial, the short or long tone was played to the subject. Immediately after the end of the tone, the correct lever was introduced until a response was given or until 60 s passed without a response. Intertrial intervals varied between 10 and 20 s (mean duration 15 s). Response latencies decreased progressively. At the end of errorless training, all available reinforcers were earned. Duration discrimination training came next, with each auditory stimulus followed by the simultaneous presentation of both response levers. The levers were simultaneously retracted after a response or 60 s. Long and short tones were presented, each with a probability of 0.50, in quasirandom order. Each session was limited to 60 trials, with intertrial intervals identical to those of errorless training. A total of 49 sessions were conducted before drug testing began. At the end of training, response accuracy reached 85-95%, depending on the subject.

Drug testing. On the basis of the stability and reliability of the drug effects, a dose of 10 mg/kg was injected during one block of five consecutive sessions. This repeated injection phase was preceded and followed by control blocks (five sessions each) with distilled water injection. Finally, one single dose of 20 mg/kg was given on the last session of the experiment. All other conditions were identical to those from Experiment 1.

Behavioural measures and data analysis. Two dependent variables were recorded: first, choice accuracy (i.e., the percentage of reinforced responses); second, response latencies following the presentation of the short and long auditory stimuli. Four blocks of five sessions were taken into account: successively, without injection (A), with vehicle injection (B),

with drug injection (C) and, finally, with vehicle injection (D). These data were subjected to an ANOVA for repeated measures and the block factor as classification criterion. Post-hoc pairwise comparisons between blocks were computed with Student's *t*-test for related samples. Performance after the single 20-mg/kg injection was compared to the average performance of the last three sessions of block D (i.e. D'), with an ANOVA for repeated measures and treatment as classification criterion. For this last set of analyses, *n* was reduced to 9.

A more refined analysis of choice accuracy took responses given after the short and the long stimulus into account separately. A first ANOVA for repeated measures and treatment as classification criterion compared the average data from the last three session before drug administration and data recorded after the first injection of the dose of 10 mg/kg. It checked for intrastimulus shifts in proportions of correct responses after drug injection. A second ANOVA for repeated measures and stimulus duration as classification criterion compared intradose differences between the proportion of correct responses given after the long and the short stimulus. It checked for an interstimulus bias in the proportion of correct responses.

Results

The top panel of Figure 7 depicts the choice accuracy. As can be seen, the drug did not impair the accuracy of the temporal discrimination, which in all cases remained close to 90%. No significant difference could be found. Separate analysis of choice accuracy after the long and the short stimulus yielded no significant results. The drug did not induce intrastimulus shifts in the proportions of correct responses. Furthermore, these proportions were similar after the short and long stimuli.

The middle and bottom panels of Fig. 7 present corresponding response latency data. The middle of the figure shows response latencies after the short stimulus; the bottom presents latencies recorded after the long stimulus. Rats reacted immediately to the protrusion of the levers. However, for mechanical reasons, a response could be recorded only from 0.3 s after the protrusion process began, which explains the extremely short response latencies. Indeed, during this delay, rats were already engaged in the initial phase of the movement before hitting the lever with their paw. Response latencies after the short stimulus (middle) were on average longer than those recorded after the long tone (bottom). They decreased after the injection of a dose of 10 mg/kg of amineptine (comparisons B/C). After 20 mg/kg, they tended to increase (comparisons D'/E). The ANOVA revealed that the block effect was significant only for response latencies after the short stimulus [$F(3, 27) = 3.75, p < 0.03$]. Pairwise comparisons yielded significant differences between blocks A/C [$t(9) = 2.997, p < 0.02$] and B/C [$t(9) = 1.916, p < 0.05$]. The small lengthening of response latencies after 20 mg/kg was close to significance only after the long auditory stimulus [$F(1, 8) = 4.66, p < 0.06$].

Discussion

As expected, amineptine at 10 and 20 mg/kg did not change the accuracy of discrimination between stimulus durations of 2 and 8 s. For the information-processing model of the timer (5), these doses of the drug thus perhaps altered neither the representation of the duration of the auditory stimuli, nor the decision rule adopted by the subjects (to press the right or left lever, after comparison between the just-presented stimulus duration stored in working memory and the represen-
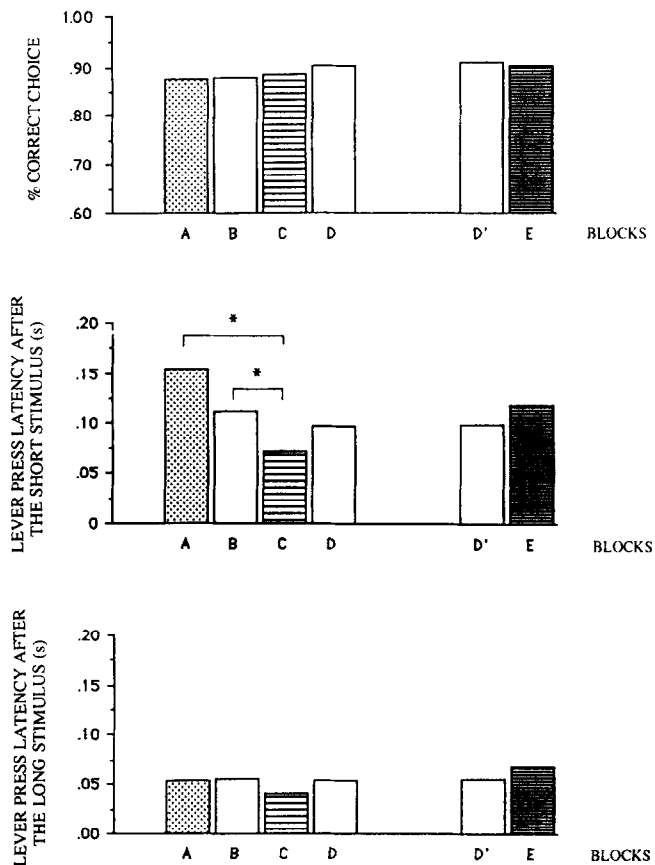

FIG. 7. Top: Effect of amineptine on the percent of correct choices (ordinate) of rats subjected to the auditory duration discrimination procedure. Except for column E, which presents data from one single session, columns are averages over blocks of five (A, B, C, D) or three sessions (D'). From the left to the right, chronologically: data without injection (A), after vehicle injection (B), after repeated amineptine injection at a dose of 10 mg/kg (C), after vehicle injection (D and D'), and finally after the single injection of 20 mg/kg (E). Middle: Effects of amineptine on lever press latencies (ordinate) following the short auditory stimulus (other details as at the top). Bottom: Effects of amineptine on lever-press latencies (ordinate) following the long auditory stimulus (other details as at the top). Significant pair comparisons: *$p < 0.05$.

tation in reference memory of the short and long stimuli). A change in clock speed might also be ruled out, as no systematic bias in choice accuracy could be detected after the very first injection of the drug. The hypothesis of a dopaminergic involvement in clock speed emerged from data described by Meck (18) using a bisection procedure and drugs modulating catecholamines, mainly dopamine. Testing under amphetamine rats previously trained without drug led to a leftward shift, whereas testing under haloperidol (a neuroleptic) produced a rightward shift of the bisection point. The former effect was interpreted as depending on a drug-induced acceleration, the latter on a drug-induced deceleration of clock speed. As amineptine is believed to induce dopamine release and dopamine uptake blockade (as does amphetamine), a bias toward the lever associated with the short stimulus might have been expected. Provided that the present results might be replicated, they would discard speeding of the internal pacemaker as a tentative explanation of the response timing results described.

Duration discrimination data tended to confirm the hypothesis derived from the first experiment: Amineptine at 10 or 20 mg/kg does not seem to modulate the timing mechanism proper, but rather acts at a more peripheral level (i.e., on the activity and vigilance of the subjects). Significant response latency decreases recorded after the injection of 10 mg/kg further support the hypothesis of an aspecific stimulating effect. Put more generally, the dissociation between response timing and duration discrimination data support a view according to which the injection of a drug modulating the dopaminergic system does not systematically target the timing mechanism proper. Drug effects might be limited to subordinate processes involved in the behavioral expression of timing, such as the ability to inhibit operant behavior.

In other respects, the difference between the absolute duration of response latencies after long and short stimuli is worth commenting on, because it highlights the response strategy followed by most of the subjects. Informal observation of the animals indicated that at each stimulus onset, the rat quickly moved to face the location of the short lever. When the stimulus ended after a short duration, the rat pressed the lever immediately in front of it. When the stimulus duration exceeded some threshold, the subjects moved to face the long lever and pressed it as soon as it was accessible. In other words, the onset of each stimulus was associated with a reinforcement opportunity of 0.5 for a short as well as long response. This ambiguity led to a longer decision time, and thus to a longer response latency, when the stimulus ended after the short duration. A long response arose at the end of the decision chain, after the choice was already made by the rat. Response latency was thus shorter after the 8-s than after the 2-s stimulus. This might also explain why after the long stimulus, latency decreases consecutive to drug injection were smaller and nonsignificant: Response latencies were already close to the lowest possible asymptotic value before administration of the drug.

This response strategy is in line with the hypothesis according to which subjects truly measure and compare durations (5). The moment of position shift from the short lever to the long one (which could not be recorded) might be considered the point of subjective equality between the long- and short-stimulus durations. This response strategy further confirms the fact that animals try to optimize performance (increase the rate of reinforcement) by adopting a time-saving response strategy. This sophisticated response process was not altered by amineptine at the doses used in the present experiment.

GENERAL DISCUSSION

The preceding experiments were designed to clarify the effects of amineptine with regard to the timing mechanism of rat subjects. Results collected thus far tend to cause us to discard the hypothesis of a direct effect of the drug on the timing mechanism proper. Indeed, the decrease in the temporal accuracy of behaviour found in DRL and FI could not be generalized to duration discrimination performance. In the latter task, rats matched baseline values after 10 or even 20 mg/kg of amineptine. Data recorded on DRL and FI might instead be due to a nonspecific stimulating effect of the drug on motor behaviour. Such an interpretation is akin to previously reached conclusions (4,35), according to which amineptine enhances spontaneous motor activity in mice and rats. The accurate duration discrimination of rats after 20 mg/kg of amineptine is also congruent with this analysis. Indeed, the absence of a response opportunity during the signal (retracted levers) prevented the expression of the motor component of the drug effect (clearly present in FI and DRL), to the benefit of the expression of an unimpaired estimation of time. Results further show that the enhancement of activity is not limited to spontaneous activity, but also concerns acquired operant activity. Such a stimulating effect was also recorded in DRL after other DA uptake inhibitors such as nomifensine (20) or bupropion (33). However, on the discrete trial waiting performance used with dogs (1), amineptine-induced motor stimulation did not achieve statistical significance (probably because this type of schedule is insensitive to such effects).

Another drug effect ensues from data recorded on CRF-S^D and duration discrimination: a trend toward an increased vigilance and reactivity to extraneous significant stimuli. This effect was expressed by a shortening of the response latency to the signal (CRF-S^D) and the protrusion of levers inside the cubicle (duration discrimination). Finally, the sophisticated cognitive decision process, as well as the elaborate response strategy in the duration discrimination task, were not altered after 10 or even 20 mg/kg of the drug. This last set of data strongly suggests that these doses of amineptine are not detrimental to cognitive parameters of behaviour, at least in rodents. Nevertheless, the present data do not imply that the drug acted as a cognitive enhancer, as was suggested in dogs (1). This difference might be related to species-specific (dogs vs. rats) or procedural variables (drug administration per os vs. IP, and so forth) whose involvement is not yet correctly understood.

On the whole, our data obtained with rodents seem to be compatible with the hypothesis according to which antidepressant and motor stimulation effects of amineptine might share common neurologic substrates (4). They substantiate neither the timing nor the cognitive enhancer hypotheses.

REFERENCES

1. Bruhwyler, J.; Chleide, E.; Rettori, M. C.; Poignant, J. C.; Mercier, M. Amineptine improves the performance of dogs in a complex temporal regulation schedule. Pharmacol. Biochem. Behav. 45:897–903; 1993.
2. Ceci, A.; Garattini, S.; Gobbi, M.; Mennini, T. Effect of long term treatment on pre- and postsynaptic mechanisms in rat brain. Br. J. Pharmacol. 88:269–275; 1986.
3. Chagraoui, A.; Vasse, M.; Protais, P. Interaction of amineptine with agents modifying dopaminergic transmission. Clin. Neuropharmacol. 12:S19–S31; 1989.

4. Chagraoui, A.; Vasse, M.; Protais, P. Effects of repeated treatments with amineptine and desipramine on motor responses involving dopaminergic systems. Psychopharmacology 102:201–206; 1990.
5. Church, R. M. Properties of the internal clock. In: Gibbon, J.; Allan L., eds. Timing and time perception, vol. 423. New York: Annals of the New York Academy of Sciences; 1984:566–582.
6. Fry, W.; Kelleher, R. T.; Cook, L. A mathematical index of performance on fixed interval schedule of reinforcement. J. Exp. Anal. Behav. 3:193–199; 1960.

7. Hand, T. H.; Marek, G. J.; Jolly, D. C.; Seiden, L. S. Antidepressant-like effects of the 5-HT$_{1A}$ agonists buspirone, gepirone, 8OHDPAT, and 5MEODMT in rats on the DRL 72 sec schedule: Differential blockade by methysergide and purported 5-HT$_{1A}$ antagonists. Soc. Neurosci. Abst. 15:1282; 1989.

8. Hicks, R. E. Prospective and retrospective judgments of time: A neurobehavioral analysis. In: Macar, F.; Pouthas, V.; Friedman, W. J.; eds. Time, action and cognition: Towards bridging the gap. Dordrecht: Kluwer Academic Publishers; 1992:97–108.

9. Iversen, S. D.; Iversen, L. L. Behavioural pharmacology. New York: Oxford University Press; 1975.

10. Kelly, J. B.; Masterton, B. Auditory sensitivity of the albino rat. J. Comp. Physiol. Psychol. 91:930–936; 1977.

11. Kemali, D. Etude multicentrique de l'amineptine (Survector 100) en Italie. Clin. Neuropharmacol. 12:S44–S53; 1991.

12. Lemoine, P.; Achaintre, A.; Balvay, G.; Bonnet, H.; Carrier, C.; Perrin, J. Double-blind trial of amineptine and clomipramine in the treatment of depression. Current Med. Res. Opin. 7:234–240; 1981.

13. Marconi, P. L.; Brugnoli, R.; Bressa, G. M. La dépression des personnes âgées traitées par l'amineptine (Survector 100). Clin. Neuropharmacol. 12:S102–S109; 1991.

14. Marek, G. J.; Seiden, L. S. Selective inhibition of MAO-A, not MAO-B, results in antidepressant-like effects on DRL 72-sec behavior. Psychopharmacology 96:153–160; 1988.

15. Maricq, A. V.; Church, R. M. The differential effects of haloperidol and metamphetamine on time estimation in the rat. Psychopharmacology 79:10–15; 1983.

16. Maricq, A. V.; Roberts, S.; Church, R. M. Metamphetamine and time estimation. J. Exp. Psychol. Anim. Behav. Proc. 7:18–30; 1981.

17. McGuire, P. S.; Seiden, L. S. Differential effects of imipramine in rats as a function of DRL schedule value. Pharmacol. Biochem. Behav. 13:691–694; 1980.

18. Meck, W. H. Selective adjustement of the speed of the internal clock and memory processes. J. Exp. Psychol. Anim. Behav. Proc. 9:171–201; 1983.

19. O'Donnel, J. M.; Seiden, L. S. Effects of monoamine oxydase inhibitors on performance during differential reinforcement of low response rate. Psychopharmacology 78:214–218; 1982.

20. O'Donnel, J. M.; Seiden, L. S. Differential-reinforcement-of-low-rate 72-s schedule: Selective effects of antidepressant drugs. J. Pharmacol. Exp. Ther. 224:80–88; 1983.

21. Offermeier, J.; Potgieter, B.; Dupreez, H. Z.; Meiring, P. J. Studies on the pharmacology of a new antidepressant, S1694. S. Afr. Med. J. 51:62–66; 1977.

22. Poignant, J. C. Revue pharmacologique sur l'amineptine. L'Encéphale 5:709–720; 1979.

23. Poirier, J. P.; Rolland, Y.; Arnaud, J. C.; Krikorian, A. Relation structure activité et mécanisme d'action neurochimique des antidépresseurs: Etude des dérivés tricycliques de l'acide amino-7-heptanoïque. Actualités Psychiatr. 7:148–153; 1980.

24. Ponzio, F.; Achilli, G.; Garattini, S.; Parego C.; Sacchetti, G.; Algeri, S. Amineptine: Its effect on the dopamine system of rats. J. Pharmacol. 38:301–303; 1986.

25. Porot, M.; Gerstner, C.; François, M. A. Activité antidépressive de l'amineptine: Etude contrôlée à double insu. Thérapie 35:733–742; 1980.

26. Richards, J. B.; Sabol, K. S.; Jolly, D. C.; Seiden, L. S. Differential effects of 5-HT$_{1A}$ agonists 8ODHPAT, buspirone, gepirone, ipsapirone and 5MEODMT on a DRL 72 sec schedule of reinforcement. Soc. Neurosci. Abstr. 16:752; 1990.

27. Richards, J. B.; Sabol, K. E.; Seiden, L. S. DRL interresponse-time distributions: Quantification by peak deviation analysis. J. Exp. Anal. Behav; 60:361–385; 1993.

28. Richards, J. B.; Seiden, L. S. A quantitative interresponse-time analysis of DRL performance differentiates similar effects of the antidepressant desipramine and the novel anxiolytic gepirone. J. Exp. Anal. Behav. 56:173–192; 1991.

29. Richelle, M.; Lejeune, H., eds. Time in animal behaviour. London: Pergamon Press; 1980.

30. Ropert, R.; Barte, H. N.; Ostapzeff, G.; Kamoun, A. Etude des effets thymo-analeptiques et de l'acceptabilité de l'amineptine comparés à ceux de l'imipramine. L'Encéphale 7:389–403; 1982.

31. Samanin, R.; Jori, A.; Bernasconi, S.; Morpugo; E.; Garattini, S. Biochemical and pharmacological studies on amineptine (S1694) and (+) amphetamine in rat. J. Pharm. Pharmacol. 29:555–558; 1977.

32. Scuvée-Moreau, J.; Dresse, A. Effect of amineptine on the firing rate of central monoaminergic neurons in the rats. Arch. Intern. Physiol. Biochim. 90:371–375; 1982.

33. Seiden L. S. Effects of trazadone, fluoxetine and bupropion on rats responding under a schedule of differential-reinforcement-of-low-rate 72 sec (DRL 72 sec). Fed. Proc. 42:1163; 1983.

34. Seiden, L. S.; O'Donnel, J. M. Effects of antidepressant drugs on DRL behaviour. In: Seiden, L. S.; Balster, R. L., eds. Neurology and neurobiology, vol. 13. Behavioural pharmacology: The current status. New York: Liss; 1985:323–338.

35. Van Amerongen, P. Double-blind clinical trial of antidepressant action of amineptine. Curr. Med. Res. Opin. 6:93–100; 1979.

36. Vauterin, C.; Bazot, M. A. double-blind controlled trial of amineptine vs. trimipramine in depression. Curr. Med. Res. Opin. 6:101–106; 1979.