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The effect of chlordiazepoxide on acquisition and extinction responding for rewarding brain stimulation.

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The Effect of Chlordiazepoxide on Acquisition
and Extinction Responding For Rewarding
Brain Stimulation

A Thesis Presented

by

Ronald Gandelman

Submitted to the Graduate School of the
University of Massachusetts in
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Abstract

Rats administered chlordiazepoxide during extinction of a bar pressing response for rewarding brain stimulation showed greater resistance to extinction than did rats administered the drug during acquisition or not given it at all. The subjects injected during both acquisition and extinction made the greatest number of extinction responses. The results were discussed in terms of a frustration hypothesis.

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AND EXTINCTION RESPONDING FOR REWARDING
BRAIN STIMULATION

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The rewarding property of electrical stimulation of the brain (ESB) is in some ways similar to and in some ways different from that of conventional rewards such as food and water. In general, similarities derive from the fact that ESB can reinforce various kinds of behavior (e.g. bar pressing, running, attack) as do conventional rewards. The differences, however, are of a more specific nature. They are as follows: i. rapid extinction of a response previously rewarded with ESB (Herberg, 1963; Olds, 1955; Olds and Milner, 1954; Seward, Uyeda, and Olds, 1959) ii. rapid reacquisition of that response (Olds, 1955; Olds and Milner, 1954) iii. lack of satiation effects with continuously rewarded ESB (Olds and Milner, 1954), although telencephalic placements (e.g. septal area) do show a decrement of responding after 4 to 8 hours (Olds, 1958a).

In an attempt to account for the disparity between the rewarding effects of ESB and conventional reinforcers, Deutsch and Howarth (1963)¹ suggested that an application of electrical stimulation to a "rewarding" area of the brain directly stimulates both motivational and reinforcement systems in the brain simultaneously.¹ Thus, ESB rewards a response and provides motivation for the next one. This explanation accounts for both the rapid

acquisition and reacquisition seen under ESB. Since the two systems are stimulated directly while no consummatory response is needed, the delay of reinforcement is eliminated as are any modulating properties of the periphery which might reduce the effectiveness of an ordinary reinforcer. Because the motivational pathway is stimulated with every reinforcement no satiation effects will be produced. Finally, because motivation is assumed to decay rapidly following the removal of ESB, extinction is rapid. The "dual process" theory of ESB can account, in theory, for the differences between the rewarding properties of ESB and conventional rewards.

Direct evidence for the dual process theory was obtained by Howarth and Deutsch (1962) who showed that extinction is a function of the time since the last reinforcement rather than of the number of nonreinforced responses. Thus, extinction without responding could be obtained. This, obviously, supports the contention of rapidly decaying motivation accrued from the electrical stimulus. Further support is given by Gallistel (1966, 1967). Employing a runway situation, he showed that an increase in the inter-trial-interval (ITI) from 5 to 60 sec. leads to an immediate decrease in running speed

while a decrease in the ITI from 60 to 5 sec. leads to an immediate increase in running speed. These results were interpreted as indication that an animal subjected to a shift in ITI from 5 to 60 sec. has a concomittant decrease in post-shift motivation since the motivation accrued from the previous electrical stimulus has longer to decay. Conversely, those subjects shifted from 60 to 5 sec. are, during the post-shift period, under increased motivation since the motivation has less time to decay. Finally, Deustch and Howarth (1962) demonstrated that a habit originally learned for ESB can be evoked solely by other natural drives. This seems to indicate that the motivation supposedly accrued from ESB is related to other naturally occuring forms of motivation.

Recent evidence, however, indicates that the rewarding properties of ESB and conventional rewards are similar and that differences between them can be explained by taking into account intrinsic differences between the two types of reward (e.g. mode of delivery) as well as evoking well established behavioral laws which have been derived to deal with conventional rewards. For example, Gibson, Reed, Sokai, and Porter (1965) proposed that the difference between ESB and conventional rewards is due to

the response-reward contingencies associated with the comparison of the two rewards. In the usual comparison, bar pressing for ESB is contrasted with bar pressing for an ordinary reinforcer. However, bar pressing can be considered the consummatory response for ESB whereas it is the instrumental response for a reward such as sucrose solution. When the contingencies were equated, i.e., rats received both ESB and sucrose immediately at a dipper or received both rewards at the dipper after making a bar press, rates of responding and resistance to extinction for subjects rewarded with ESB and sucrose did not differ. Most studies concerned with the rapid extinction of a response previously rewarded with ESB are plagued with another confounding artifact. Usually, ESB rewarded subjects are maintained under free feeding conditions whereas conventionally rewarded animals are, for the most part, deprived during testing. It is well known (Soltzman and Koch, 1948; Strassberger, 1954; Yarnaguchi, 1951) that greater resistance to extinction will be exhibited by animals maintained on a more extreme deprivation schedule than will be shown by moderately deprived animals. Thus the lack of deprivation-induced drive may contribute to the rapid extinction of an ESB

rewarded response. Finally, Fanksepp and Trowill (1967) showed that satiated rats administered a highly palatable solution of chocolate milk directly into their mouths as a reward for bar pressing, exhibit behavior similar to animals rewarded with ESB (rapid acquisition and extinction, extinction without responding).

The following experiment will be concerned with the rapid extinction of an ESB rewarded response. As mentioned above, one possible contributor to rapid extinction may be the satiated condition of ESB rewarded subjects. Deutsch and DiCara (1967) do show that hungry animals responding for ESB exhibit greater resistance to extinction than do rats maintained on free feeding schedules. (It must be noted that only those hungry subjects which show an increase in the rate of responding for ESB exhibit an increase in resistance to extinction). Lack of deprivation-induced drive, however, does not seem to be the sole cause of rapid extinction, for it is paradoxical that such rapid extinction can follow behavior characterized by rapid acquisition, lack of satiation, and exceedingly high rates of responding (usually on the order of 40 - 80 responses per minute when electrodes are placed within the lateral hypothalamus). It is likely

that another factor is involved. The following is an attempt to identify that other contributor to rapid extinction.

It is hypothesized that nonreinforced responses for ESB generate more frustration than does nonreinforcement of conventional rewards. Evidence that ESB is of a greater incentive magnitude than are conventional rewards lends credence to this proposal. Olds (1958b) reported that rats will accept more foot shock in order to obtain ESB than they will for a food reward. Falk (1961) showed that rats will, up to extreme water deprivation conditions (96 hours), respond on a bar which delivers ESB and thus ignore a second bar which delivers water. Finally, rats with electrodes in the medial forebrain bundle will starve themselves when given the opportunity to bar press for ESB during the daily one hour feeding session (Routtenberg and Lindy, 1965).

There is evidence which indicates an inverse relationship between amount of reward and resistance to extinction (Armus, 1959; Hulse, 1958; Wagner, 1961; Zaretsky, 1965). This evidence substantiates the common sense notion that more frustration is elicited by the nonreinforcement of large rather than small rewards.

Although one cannot consider ESB as being of a quantitatively greater magnitude than more typical rewards, it is compelling to view ESB as being of an enhanced quality (i.e. possessing more incentive value than conventional rewards). According to Wilson, Weiss, and Amsel (1955) nonreinforcement elicits more frustration as the development of $r_R - s_R$ (fractional anticipatory reward) increases. Similarly, Amsel and Hancock (1957), using the double runway situation, presented evidence in favor of a positive relationship between the frustration effect and the strength of the anticipatory goal response preceding the nonreward. It was found that running speed increased more in the second alley after nonreward in the first goal box when the two runways were similar. If, due to similarity of cues, one considers the fractional anticipatory goal response to be greater in the second runway when the two runways are similar, Amsel and Hancock's interpretation appears tenable. Accepting, as does Spence (1956) a synonymy between fractional anticipatory reward and incentive, it follows that the greater incentive properties of ESB can cause a nonreinforced, ESB-directed response to generate more frustration than one previously rewarded in a more conventional manner. Since frustration

allegedly provides the drive for competing responses (Ansot, 1962), one would expect rapid extinction of a response previously maintained by DAB.

It is necessary, in order to test the above hypothesis, to show an inverse relationship between resistance to extinction and frustration. Berry, Wagner, and Miller (1962) presented evidence which indicated that the administration, during extinction, of drugs which have formerly been shown to improve the performance of a response previously inhibited by fear, leads to an increase in resistance to extinction. The authors interpreted the results as indicating that these drugs, alcohol and sodium amytal, reduced frustration accrued from nonreinforcement. Chlordiazepoxide (librium), a tranquilizer, was similarly shown to facilitate the performance of a response which had been previously inhibited by fear (Feldman, 1962; Lewis and Feldman, 1964). In these studies a Maier-type paradigm was used. This procedure is described by Feldman and Green (1967; p. 250) as follows:

The Maier paradigm is a two-stage procedure based on two-choice discrimination methodology using the Lashley jumping stand. In the first stage the two-choice problem is insoluble - half the responses to any cue are randomly rewarded and half are punished (hitting a locked window and falling to a net below) for 160 trials - 10 trials a day. The behavioral result is

usually a stereotyped response to a position. In the second stage the problem is made soluble. A position stereotyped animal in this stage usually has a non-spatial cue (dark) designated as correct. In a typical experiment about 15-20% of the rats do solve the problem, but the rest persist in the stereotypes formed during the prior insoluble problem for a 200-trial duration of the soluble problem. (parenthesis added).

It must be noted that there is another source of punishment in this task aside from hitting the locked window and falling to a net. All animals are given goading foot shock while on the jumping stand if they do not respond within a specified length of time. Feldman (1962) showed that 78% of the rats administered librium during the insoluble problem phase solved the problem during the solvable stage. The results were interpreted as indicating that librium decreased the strength of negative incentives (i.e. the fear of punishment). Therefore, librium might, when administered during extinction of a response previously rewarded with ESB, reduce frustration and thus lead to enhanced resistance to extinction.

A 2 X 2 factorial design was used to test the prediction. Subjects were run under the 4 possible conditions of being trained and/or extinguished with or without

the drug.

Method

Subjects

Sixteen naive, male, Charles River albino rats, approximately 90 days old at the time of electrode implantation were individually housed and maintained on a regimen of ad libitum food and water. All met the criterion for self-stimulation imposed during screening (see below).

Surgery

The Rs were anesthetized with sodium nembutal. Two stainless steel monopolar electrodes, 0.25 mm in diameter, were stereotactically implanted. Identical coordinates (Krieg, 1946) of 1.7 mm posterior to bregma, 1.4 mm lateral to the midline, and 8.2 mm below the level of the skull were used for each electrode. The coordinates were chosen so as to establish placement in the lateral hypothalamus. All Rs were permitted 5 days of recovery before experimental manipulation began.

Apparatus

Tests were run in an 18 X 24 X 18 in. high box. The front wall consisted of clear Plexiglass while the back and one side wall were of Masonite. The second side wall,

upon which a model 1352 Lohigh Valley permanent lever was mounted, consisted of galvanized sheet metal. The floor was made of wire mesh. Standard relay equipment permitted S to receive stimulation concurrent with a bar press. Each response was recorded on a digital counter. Responses per minute were recorded manually.

Stimulation

The stimulation was 0.5 sec. of 60-cycle sine-wave current delivered between one of the implanted electrodes and an indifferent surface screw. The current was regulated by a micropot used as a voltage divider and was monitored by an AC microammeter in series with S.

Screening and Habituation

On the 6th day following surgery S was allowed 10 min. of free exploration of the box after which screening was initiated. The S was given 0.5 sec. bursts of stimulation in increasing steps of 50 μ until signs of increased exploration and activity were observed. Stimulation was further increased until S could be trained to remain in one corner of the box. The terminal level of stimulation was that intensity to which S responded; i.e. remained in the appropriate corner of the box. If an S failed to remain in the designated corner with an intensity of 200 μ

the procedure was repeated with the second electrode. If the stimulation was still ineffective S was discarded. Final intensities ranged from 35 to 120 μ . (Table 1 gives the final intensities used for each S).

The Ss were randomly divided into the following four groups: no drug during acquisition - no drug during extinction (ND-ND); no drug acquisition - drug extinction (ND-D); drug acquisition - drug extinction (D-D); drug acquisition - no drug extinction (D-ND). A week after screening all Ss were given three consecutive days of habituation to librium. Each day, in their home cages, Ss received an intraperitoneal injection of 15 mg/kg, the dosage used throughout the experiment. (This was also the dosage used by Feldman, 1962). On the fourth day Ss were allowed 30 min. of free exploration of the box with groups D-D and D-ND receiving the drug before placement in the apparatus. (On drug days all Ss were injected 20 min. before placement in the box). Two days later acquisition was begun.

Acquisition and extinction

The Ss were run through the following manipulations in squads of four, one from each group.

Table 1

HD-ND		HD-D		D-D		D-ND	
<u>S</u>	<u>μ_a</u>	<u>S</u>	<u>μ_a</u>	<u>S</u>	<u>μ_a</u>	<u>S</u>	<u>μ_a</u>
10	70	11	115	13	45	12	75
15	120	16	45	18	40	17	35
19	80	20	50	26	110	23	85
27	65	28	35	30	45	29	100

Intensities (μ_a) used for each subject (S).

Day 1.

The Ss were trained to bar press for ESB. The session was terminated after S met the criterion of 1,800 responses. If 3 min. of no responding occurred, sufficient priming was given to reestablish the response. The amount of time needed to reach criterion was recorded.

Day 2.

Same as Day 1 with the exception that S was given 3 min. from the time of placement in the box in which to respond. If S did not respond, sufficient priming was given to initiate responding.

Day 3.

The Ss remained in their home cages. No injections were given.

Day 4.

The Ss were permitted 90 reinforced responses after which they were placed on extinction. The extinction session lasted for one hour with the number of extinction responses recorded.

Results

The total times to criterion in acquisition (Day 1 + Day 2) for the groups receiving librium and for those not

receiving the drug were 551 min. and 574 min. respectively. A Mann-Whitney U test² indicated no significant difference in time to acquisition criterion between the two groups ($U=30$, $p>.4$).

Significant differences, however, were found in resistance to extinction. Table 2 gives the number of extinction responses made by each S while table 3 gives the mean number of extinction responses and the range of responses exhibited by each group. Group D-D made significantly more extinction responses than did any of the other groups ($U=0$, $p<.02$ between all combinations). Group MD-D made more responses than did groups MD-MD and D-MD ($U=1\frac{1}{2}$, $p<.05$ and $U=0$, $p<.02$ respectively). There was no significant difference between groups MD-MD and D-MD ($U=8$, $p>.4$).

Discussion

It is apparent that the animals receiving librium during nonreinforcement showed greater resistance to extinction than did those not receiving the drug during extinction. Thus, to the extent that librium reduces frustration, the data support the hypothesis that frustration is a factor responsible for the rapid extinction of

Table 2

ND--ND		ND--D		D--ND		D--D	
<u>S</u>	<u>Rs</u>	<u>S</u>	<u>Rs</u>	<u>S</u>	<u>Rs</u>	<u>S</u>	<u>Rs</u>
10	31	11	53	12	41	13	97
15	13	16	54	17	16	18	145
19	36	20	57	23	47	26	95
27	54	28	57	29	18	30	159

Total number of responses (Rs) given by each subject.

Table 3

<u>Group</u>	<u>Mean</u>	<u>Range</u>
ND-ND	33.5	13-54
ND-D	55.2	53-57
D-ND	30.5	16-47
D-D	124.0	95-159

Mean number of extinction responses given by each group and the range of responses within each group

a continuously reinforced response for ESB.

The frustration hypothesis might not appear compatible with the significant difference in resistance to extinction between groups ND-D and D-D. Since both groups were given the drug during a period in which frustration presumably occurred, there should have been no difference between them. A stimulus change effect can, however, account for this apparent discrepancy. Belleville (1964) showed that resistance to extinction was greater when extinction occurred under the same conditions (drug or no drug) as existed during acquisition. In the present study, group ND-D received stimuli in extinction which differed, in part, from those during acquisition. It is likely that the change from no drug acquisition to drug extinction produced stimulus generalization decrement and thus caused a decrement in extinction responding for group ND-D. It can be asked why there is no similar decrement in extinction responding for group D-ND as compared to group ND-ND. It appears that both groups extinguished so rapidly that the shift from drug to no drug conditions of group D-ND was not powerful enough to cause a decrement.

The hypothesis that the rapid extinction of an ESB

rewarded response is in part a function of the frustration accrued during extinction finds added support in the findings of Herberg (1963) and Pliskoff and Hawkins (1963). Herberg (1963) found greater resistance to extinction for subjects rewarded with ESB on a fixed ratio (FR) schedule of reinforcement than for continuously reinforced subjects. It is likely that the FR subjects were simply trained to respond in the face of frustration (nonrewarded responses). From the results of this investigation it is predicted that animals given librium when on FR schedules will exhibit less resistance to extinction than animals subjected to FR schedules when not drugged. The former will not be trained to respond in the face of frustration since no appreciable amount of frustration will exist. Pliskoff and Hawkins (1963) did not obtain extinction without responding when the lever was intermittently withdrawn during training. Again, the subjects were taught to approach and resume responding in the presense of frustration (the blocking of the instrumental response via lever withdrawal). The effect of frustration in the ESB situation can, therefore, be overcome by appropriate training.

In the present study, frustration appeared to have had

two effects on subsequent responding. At the onset of extinction, the frustration had motivational properties similar to those found by Ansel and Rousell (1952).

Employing the double runway paradigm, they found that running speed in alley 2 increased on those trials in which the rat was not rewarded in goal box 1. In the present study, a rapid burst of responding of an approximate duration of one minute began simultaneously with the start of extinction for all subjects. After the first minute of extinction the aversive properties of frustration were in evidence. The animal left the immediate area of the bar for the opposite side of the box, groomed excessively and appeared to sleep. The no drug extinction subjects rarely if ever returned to the bar. Most of their extinction responding occurred during the first two minutes. The drug extinction subjects, however, frequently returned directly to the bar and responded.³ Librium, therefore, had no obvious effect on the motivational component of frustration since that component was exhibited by both the drug and no drug extinction groups. However, the drug did reduce the aversive component. It appears, then, that librium, as well as depressants such as alcohol and sodium amytal, can be used to investigate

the role of frustration in the extinction situation.

In retrospect, the dual process theory of rewarding brain stimulation cannot account for the increased resistance to extinction of rats given librium during non-reinforcement. According to that theory increased resistance to extinction would necessitate preservation of the rapidly decaying motivation accrued from the electrical stimulus. However, no evidence of motivational preservation has been presented by these theorists. The position of the present author attributes the enhanced resistance to extinction to the frustration alleviating property of librium. These data suggest, then, that the rewarding property of electrical brain stimulation is comparable to other more usual types of reinforcement such that any differences between ESB and conventional rewards can be explained in terms of the enhanced incentive value of the brain stimulus. Thus, one can deal with ESB without imparting hypothetical anatomical substrates apart from those anatomical mechanisms which might be shared by all rewards.

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Footnotes

1. For a thorough review of the dual process position see Gallistel (1963) and Deutsch and Deutsch (1966).
2. All statistical comparisons were made with the Mann-Whitney U Test (Siegel, 1956).
3. The number of responses given by each subject per minute is contained in the appendix.

APPENDIX

Number of extinction responses given
by each subject per minute

APPENDIX

Group MD-ID

<u>minute</u>	<u>subject</u>			
	<u>10</u>	<u>15</u>	<u>19</u>	<u>27</u>
1	27	11	24	38
2	1	2	12	4
3	2	0	0	8
4	0	0	0	4
5	1	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0
13	0	0	0	0
14	0	0	0	0
15	0	0	0	0
16	0	0	0	0
17	0	0	0	0
18	0	0	0	0
19	0	0	0	0
20	0	0	0	0
21	0	0	0	0
22	0	0	0	0
23	0	0	0	0
24	0	0	0	0
25	0	0	0	0
26	0	0	0	0
27	0	0	0	0
28	0	0	0	0
29	0	0	0	0
30	0	0	0	0

<u>minute</u>	<u>subject</u>			
	<u>10</u>	<u>15</u>	<u>19</u>	<u>27</u>
31	0	0	0	0
32	0	0	0	0
33	0	0	0	0
34	0	0	0	0
35	0	0	0	0
36	0	0	0	0
37	0	0	0	0
38	0	0	0	0
39	0	0	0	0
40	0	0	0	0
41	0	0	0	0
42	0	0	0	0
43	0	0	0	0
44	0	0	0	0
45	0	0	0	0
46	0	0	0	0
47	0	0	0	0
48	0	0	0	0
49	0	0	0	0
50	0	0	0	0
51	0	0	0	0
52	0	0	0	0
53	0	0	0	0
54	0	0	0	0
55	0	0	0	0
56	0	0	0	0
57	0	0	0	0
58	0	0	0	0
59	0	0	0	0
60	0	0	0	0

Group HD-D

<u>minute</u>	subject			
	<u>11</u>	<u>16</u>	<u>20</u>	<u>28</u>
1	20	22	11	19
2	0	0	4	11
3	1	1	4	11
4	0	1	5	0
5	2	0	0	0
6	0	0	1	0
7	0	0	0	0
8	1	0	0	1
9	0	0	0	1
10	1	0	0	0
11	0	0	0	0
12	5	1	0	0
13	0	0	1	0
14	0	0	1	0
15	0	0	0	0
16	0	0	0	0
17	1	0	0	0
18	0	0	0	0
19	1	0	0	0
20	0	2	0	0
21	2	0	0	0
22	0	0	0	0
23	0	0	0	0
24	0	1	0	0
25	0	1	0	0
26	0	0	0	1
27	1	1	1	0
28	0	2	0	0
29	1	1	0	0
30	0	0	0	0

<u>minute</u>	subject			
	<u>11</u>	<u>16</u>	<u>20</u>	<u>28</u>
31	0	0	2	1
32	0	0	0	1
33	0	0	1	0
34	2	0	2	0
35	0	0	0	0
36	0	1	0	0
37	1	5	0	0
38	0	1	0	0
39	0	0	0	0
40	0	0	0	0
41	2	0	0	0
42	0	0	0	0
43	0	0	0	0
44	1	0	0	1
45	2	0	0	2
46	1	0	0	1
47	0	0	0	0
48	0	1	0	0
49	0	1	0	0
50	0	1	1	0
51	2	2	1	0
52	1	0	2	1
53	1	2	5	0
54	1	1	5	0
55	0	1	1	1
56	0	2	1	1
57	0	2	2	1
58	1	0	2	1
59	1	1	1	2
60	1	0	3	0

Group D-MD

<u>minute</u>	subject			
	<u>12</u>	<u>17</u>	<u>23</u>	<u>29</u>
1	24	11	31	5
2	4	1	2	0
3	3	4	4	0
4	5	0	1	8
5	5	0	4	0
6	0	0	0	4
7	0	0	1	1
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0
13	0	0	0	0
14	0	0	0	0
15	0	0	0	0
16	0	0	0	0
17	0	0	0	0
18	0	0	0	0
19	0	0	0	0
20	0	0	0	0
21	0	0	0	0
22	0	0	0	0
23	0	0	0	0
24	0	0	0	0
25	0	0	0	0
26	0	0	0	0
27	0	0	0	0
28	0	0	0	0
29	0	0	0	0
30	0	0	0	0

<u>minute</u>	subject			
	<u>12</u>	<u>17</u>	<u>23</u>	<u>29</u>
31	0	0	0	0
32	0	0	0	0
33	0	0	0	0
34	0	0	0	0
35	0	0	0	0
36	0	0	0	0
37	0	0	0	0
38	0	0	0	0
39	0	0	0	0
40	0	0	0	0
41	0	0	0	0
42	0	0	0	0
43	0	0	0	0
44	0	0	0	0
45	0	0	0	0
46	0	0	0	0
47	0	0	0	0
48	0	0	0	0
49	0	0	0	0
50	0	0	0	0
51	0	0	0	0
52	0	0	0	0
53	0	0	0	0
54	0	0	0	0
55	0	0	0	0
56	0	0	0	0
57	0	0	0	0
58	0	0	0	0
59	0	0	0	0
60	0	0	0	0

Group D-D

<u>minute</u>	subject			
	13	18	26	30
1	31	28	26	38
2	4	21	14	21
3	11	5	11	14
4	2	6	0	5
5	3	4	4	2
6	0	1	2	0
7	0	2	0	0
8	2	0	0	1
9	1	0	0	0
10	0	5	0	2
11	2	0	0	0
12	1	1	8	4
13	0	1	0	0
14	0	0	1	0
15	4	0	0	0
16	0	0	0	0
17	2	0	0	0
18	1	3	0	13
19	0	4	0	0
20	0	2	2	0
21	0	0	0	2
22	0	0	0	0
23	1	1	0	0
24	1	0	1	0
25	0	8	0	0
26	1	8	2	0
27	2	1	0	1
28	0	0	0	0
29	1	0	0	1
30	1	5	0	1

<u>minute</u>	subject			
	13	18	26	30
31	2	1	0	11
32	0	1	0	2
33	3	2	0	10
34	5	0	2	0
35	0	0	1	0
36	0	8	0	0
37	0	0	0	4
38	0	0	0	5
39	1	5	2	4
40	0	0	0	1
41	0	0	1	0
42	2	3	0	0
43	0	2	1	0
44	0	1	0	11
45	0	0	0	0
46	0	0	2	1
47	0	1	0	1
48	0	2	4	0
49	0	1	0	1
50	0	1	1	0
51	0	2	0	1
52	1	0	0	1
53	0	4	3	1
54	0	0	0	1
55	2	1	2	0
56	0	1	0	0
57	0	1	4	0
58	0	0	0	0
59	1	0	1	0
60	0	2	0	0

