

## A PARAMETRICAL STUDY OF TWO-WAY ACTIVE AVOIDANCE ACQUISITION AND LONG-TERM RETENTION: NEW APPROACHES FOR DATA ANALYSES

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The acquisition and long-term retention (LTR, 14 days) of a massed two-way active avoidance conditioning using two parameters of conditioned stimulus (CS) duration and unconditioned stimulus (US) intensity was studied. Two different tests of LTR were used: with and without the presentation of the US (Additional learning and Extinction). Results were analyzed by means of analyses of variance complemented with the use of other non-traditional statistical analyses (time-series and survival analyses). Results showed a better performance using the US of lower intensity, specially when the longer CS was used. The use of an extinction procedure does not seem to be as adequate as the use of a session of additional learning to assess LTR. On the other hand, both time-series and survival analyses can afford valuable data to complement and improve those obtained from the analyses of variance.

**Key words:** Two-way Active Avoidance; Unconditioned Stimulus Intensity; Conditioned Stimulus Duration; Time-Series Analysis; Survival Analysis; Learning and Memory.

*Estudio paramétrico de la adquisición y retención a largo plazo de la evitación activa de dos sentidos: Nuevas perspectivas de análisis.* Se estudió la adquisición y retención a largo plazo (RLP, 14 días) de un condicionamiento masivo de evitación activa de dos sentidos utilizando dos parámetros diferentes de duración del estímulo condicionado (EC) y de intensidad del estímulo incondicionado (EI). Se realizaron dos tipos de pruebas de RLP: con y sin presentación del EI (aprendizaje adicional y extinción). Los resultados fueron analizados mediante análisis de la variancia, complementados con otros análisis estadísticos no tradicionales (el análisis de la supervivencia y el de series temporales). Los resultados indican una mejor ejecución con el EI de menor intensidad, especialmente cuando el EC tiene una mayor duración. El uso de un procedimiento de extinción no parece ser tan adecuado para valorar la RLP de los sujetos como el aprendizaje adicional. Por otro lado, el análisis de series temporales y el de la supervivencia pueden aportar valiosos datos que permitan complementar y mejorar los obtenidos a partir de los análisis de la variancia.

**Palabras claves:** Evitación Activa de dos Sentidos; Intensidad del Estímulo Incondicionado; Duración del Estímulo Condicionado; Análisis de Series Temporales; Análisis de la Supervivencia; Aprendizaje y Memoria.

Two-way active avoidance is a conditioning technique widely used to study the effects of several treatments, whether behavioral or neurophysiological, upon the faci-

litation or disruption of the acquisition, retention and extinction of learning (see, for example, De Wied, 1965; Izquierdo, 1975; Ruthrich, Wetzel and Matthies, 1982; Van Hulzen and Coenen, 1982; Callen, 1986; Fernández and Coll, 1987).

Due to its wide use, relatively accurate data about some parameters influencing the level of acquisition and retention of this kind

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of conditioning are available. The effects induced by variations in the duration of the inter-stimulus interval (Low and Low, 1962; Black, 1963; Hoffman, 1966; Archer, Ogren and Johansson, 1984) and in the intensity of the electrical shock (Theios, Lynch and Lowe, 1966; McAllister, McAllister and Douglas, 1971; Tobeña, 1979; Archer et al., 1984) are specially well known. Nevertheless, few data are available concerning the variables influencing the retention of a task which has already been learned. This lack of data is of special concern if we have into account that the results reported by different laboratories have been obtained using widely different training parameters, and this fact makes it difficult their comparison and interpretation and imposes an important restriction to the analysis of the mechanisms controlling this kind of behavior (Archer et al., 1984).

In that sense, we have intended to analyze whether the conditions influencing the level of acquisition of two-way active avoidance conditioning do exert a similar influence upon its long-term retention. Specifically, we have studied the effects induced by variations in the intensity of the unconditioned stimulus (US; electrical shock) and in the duration of the conditioned stimulus (CS; tone) upon the acquisition of the task and its long-term retention (LTR; 14 days). Since in some experiments the retention of learning has been measured by means of a session of similar characteristics to those of the learning session (Van Hulzen and Coenen, 1982; Oniani and Lortkipanidze, 1985; Martí, Portell and Morgado, 1988; Segura, Capdevila, Portell and Morgado, 1988; Coll, Martí and Morgado, 1991) and, in other ones, by means of an ordinary extinction session (i.e., without the presentation of the US) (De Wied, 1965), we have decided to use both methods to evaluate LTR.

Another issue which, from our point of view, deserves special attention is the use of adequate statistical methods to analyze beha-

vioral responses. At present new statistical tools are available allowing a marked improvement of the analyses of data. Thus, although the analysis of variance affords us with undoubtedly valuable information to determine the existence of differences among the treatment groups, it has, nevertheless, some limitations when carrying out longitudinal follow-up studies in which the main variable to be analyzed is the time interval between an initial event and a final event. In the context of learning tasks, it can be of great interest to analyze the temporal evolution of their acquisition and LTR and to compare such evolution in different experimental groups. To that effect, we can use, on one hand, the survival analysis, a method used for the first time in engineering to study the resistance of materials, but that has been hardly used in psychological studies. Within a learning setting, for example, this technique makes it possible to determine the percentage of subjects which, in every phase or trial along a session, reach a predetermined performance criterion, allowing in this way to analyze the speedness of learning and to compare this speedness in different experimental groups (see, for example, Domènech, 1988 and 1989).

Another statistical technique which is still scarcely used in Psychology is time-series analysis (Gottman, 1981; Uriel, 1985). This technique arose within the field of physical sciences, but it is now beginning to be used for the analysis of behavioral studies. Briefly stated, this analysis allows the establishment of descriptive and predictive models about the temporal evolution of a given variable, both in individual subjects and in groups. In addition, it makes it possible to compare different temporal curves and to determine (with an statistical criterion) the specific moments when the curves of two different subjects or groups differ.

Having into account what has been said above, the present work has a double aim: in first place, to analyze the effects of the in-

tensity of the US and of the duration of the CS upon both the acquisition and LTR (14 days) of two-way active avoidance; and, in second place, to illustrate the application of other statistical methods complementary to the traditional analysis of variance to evidence how the analyses of results within the field of Psychology can be enriched.

## METHODS

### *Subjects*

Subjects were 58 albino male Wistar rats from our breeding stock with a mean age at the beginning of the experiment ranging from 90 to 120 days. They were subjected to controlled conditions of environmental temperature (20-25°C) and humidity (40-70%) and to a 12-12 light-darkness cycle (lights on at 8 a.m.). Water and food was available ad libitum.

### *Procedures*

Three days before the beginning of the experimental process, each animal was placed in an individual plastic cage (26×26×14 cm). Every one of those three days the animals were weighed in order to habituate them to be manipulated.

All subjects were given a single massed training session on two-way active avoidance. Just prior to this session the animals were subjected to 10 minutes of adaptation to the conditioning cage (Lafayette, LA 85150-SS), during which time neither the CS nor the US were presented. The training session consisted of 60 conditioning trials. The CS used was a tone of 80 dB and 1000 Hz. The responses (going from one compartment to the other) made during the presentation of the CS were considered as avoidance responses. In case that an avoidance response was not made, the CS was followed, with no delay, by an US which duration was of 30 seconds at most. The inter-trial interval lasted 1 minute. Subjects were randomly distributed into the 4 following groups depending on both

the intensity of the US (either 0.6 mA or 1 mA) and the duration of the CS (either 3" or 10"): 1) 0.6mA-3" (n=14); 2) 0.6mA-10" (n=14); 3) 1mA-3" (n=15), and 4) 1mA-10" (n=15).

Fourteen days after the training session, a new session (30 trials) was administered. The purpose of that session was to determine, by means of two different methods, the LTR of learning. To that effect, subjects were randomly assigned to one of the two following conditions: seven subjects in each group received a new learning session consisting of 30 trials of the same characteristics that the trials in the acquisition session (additional learning). The remaining subjects in each group were administered an extinction session of 30 trials, in which the presentation of the US was omitted (ordinary extinction). Thus, having into account the different experimental conditions, 8 groups were considered in the analysis of LTR.

*Table 1*

Summary of the experimental design used during the acquisition phase

	3" CS	10" CS
0.6 mAUS	n = 14	n = 14
1.0 mA US	n = 15	n = 15

During the acquisition and LTR sessions the kind of responses (avoidance or escape) and the latency of responses for every trial were recorded.

## RESULTS

### *Analysis of Variance*

#### *Acquisition*

Figure 1 shows the mean number of total avoidances and the mean latency of responses made by each of the 4 experimental groups during the acquisition session. As it can be seen, the different parameters used during conditioning seem to have influenced upon

the level of learning of the subjects, since there seem to be differences among groups. An analysis of variance showed that the *intensity of the US* had a significant effect upon the level of acquisition [ $F(1,54)=8.58$ ;  $p=0.005$ ], the groups trained with the lower shock intensity (0.6 mA) achieving higher conditioning levels than the groups trained with the higher shock intensity (1 mA). The analysis of latencies confirmed this effect [ $F(1,54)=5.22$ ;  $p=0.026$ ].

Neither the *duration of the CS* nor the interaction factor (*intensity \* duration*) were significant, either for the number of avoidances nor for the mean latencies of responses.

To analyze the evolution of conditioning throughout the acquisition session, this session has been subdivided into 6 blocks of 10 trials each. Figures 2 and 3 show the number of avoidances and the mean latencies of responses in each block for each experimental group.

### ACQUISITION

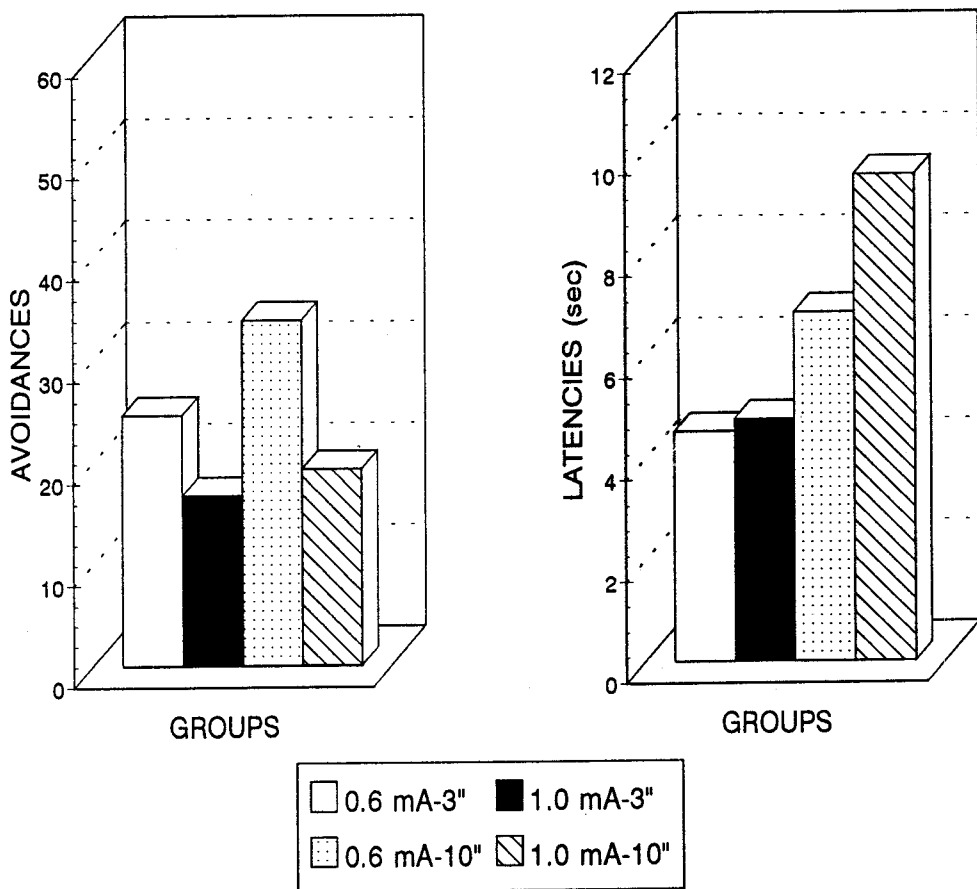


Figure 1: Performance of the different groups of subjects during the acquisition session. The left figure shows the mean number of avoidance responses, while the right figure shows the mean latencies of responses in seconds.

MEAN AVOIDANCES FOR EACH 10-TRIAL BLOCKS

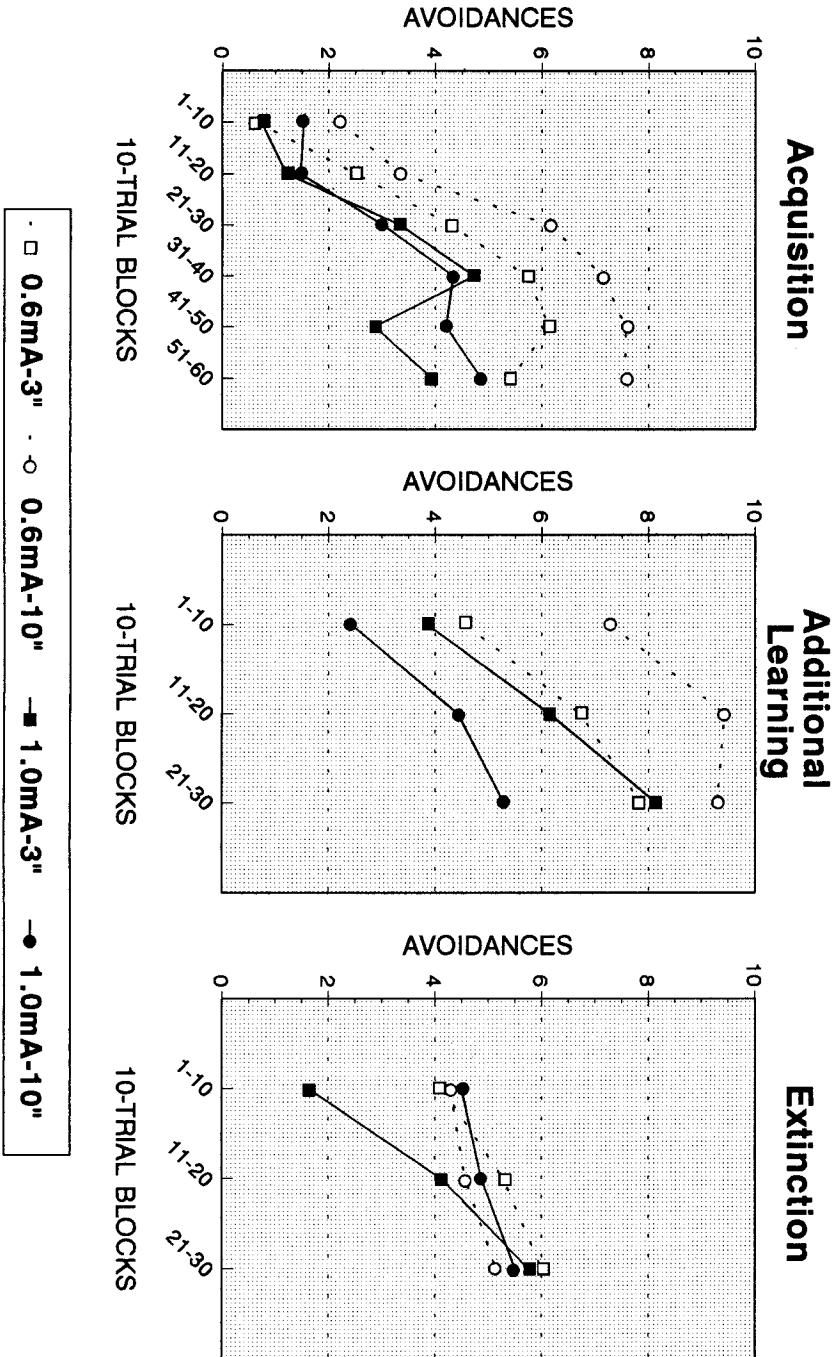


Figure 2: Mean avoidances for each 10-trial blocks composing the different experimental conditioning sessions: acquisition, additional learning and extinction.

MEAN LATENCY RESPONSES FOR EACH 10-TRIAL BLOCKS

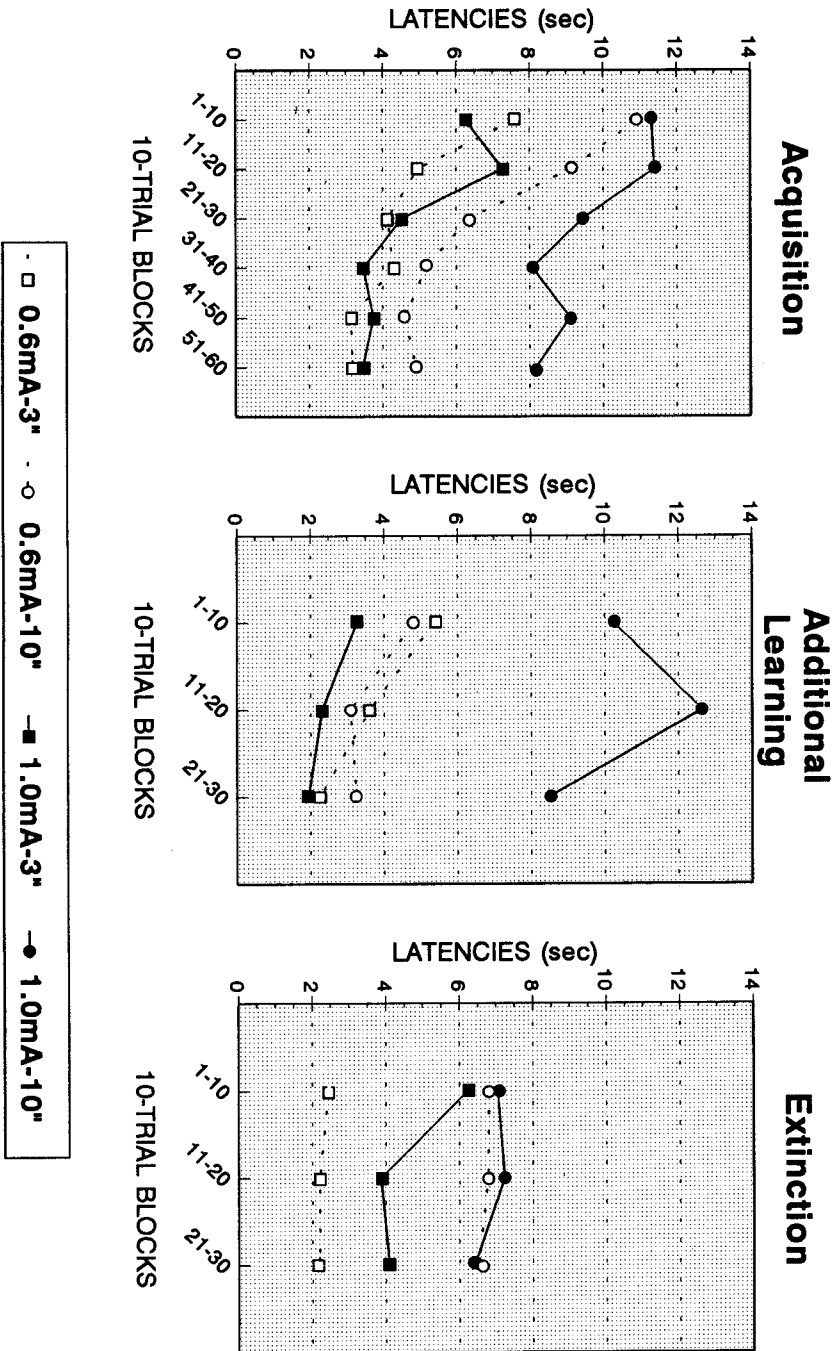


Figure 3: Mean latency of responses (in seconds) for each 10-trial blocks composing the different experimental conditioning sessions: acquisition, additional learning and extinction.

The latter analysis indicated that the interaction *block \* intensity of the US* was significant. The detailed analyses of this interaction indicated that the intensity of the US was significant only from the second block of trials on, but not on the first block.

On the other hand, a contrast analysis (Polynomial) evidenced that the evolution of the number of avoidances during the acquisition session fitted, in general terms, to an ascending linial function [ $F(1,54)=82.71$ ;  $p<0.001$ ], specially during the first 4 blocks, with a tendency to be maintained constant during the last 2 blocks. During the first 40 trials the evolution of learning depended upon the intensity of the US [interaction *block \* intensity of the US*:  $F(1,54)=4.16$ ;  $p=0.046$ ]. In general terms, those groups trained with a 0.6mA US showed an ascending linial evolution with a higher slope than the groups trained with a 1mA US [simple effects for 0.6mA and 1.0mA, respectively:  $F(1,56)=61.77$ ;  $p<0.001$ ;  $F(1,56)=26.56$ ;  $p<0.001$ ], as deduced from the "F" values. In this sense, only those groups trained with a 0.6mA US showed a significant increase in the second block compared to the first block [ $F(1,56)=14.47$ ;  $p<0.001$ ], while from trials 20 to 40 all groups showed a significant increase of performance. On the other hand, while the groups trained with a 0.6mA US showed an asymptotic evolution during the latter 20 trials, the ones trained with 1.0mA US showed a decrease from the fourth to the fifth blocks [ $F(1,56)=5.11$ ;  $p<0.028$ ] and a further increase from the fifth to the sixth blocks [ $F(1,56)=5.17$ ;  $p<0.027$ ]. The study of latencies corroborated all those results.

### Retention

Figure 4 shows both the number of avoidances and the mean latencies of response for each experimental group during the LTR test carried out 14 days after the acquisition session (additional learning and extinction).

As seen in this figure, the parameters used during the LTR test have also had an in-

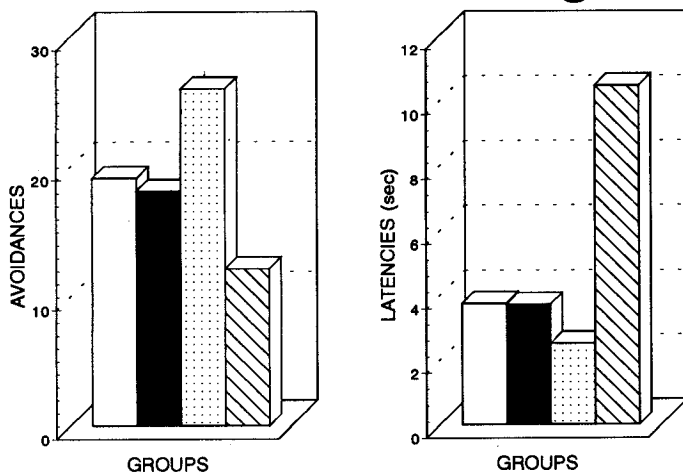
fluence upon the performance of the subjects. An analysis of variance indicated that the interaction *intensity of the US \* duration of the CS \* kind of LTR test* was significant, both when analyzing the number of avoidances [ $(1,50)=4.29$ ;  $p=0.044$ ], and the mean latencies [ $F(1,50)=7.52$ ;  $p=0.008$ ]. Further analyses indicated that the influence of both independent variables was only significant when LTR was measured by the use of an additional learning session, but not when it was measured by an extinction session.

Thus, on the additional learning session the interaction *intensity of the US \* duration of the CS* was significant both for the number of avoidances [ $F(1,50)=4.4$ ;  $p=0.041$ ] and for the mean latencies [ $F(1,50)=8.81$ ;  $p=0.005$ ]. Further analyses indicated that those subjects trained with a 0.6mA US showed higher LTR levels (both in number of avoidances and in latencies of responses) than those subjects trained with a 1.0mA US, but only when the duration of the CS was "10" [ $F(1,24)=14.24$ ;  $p=0.001$  for the number of avoidances and  $F(1,24)=10.95$ ;  $p=0.003$  for the latencies of responses, respectively]. On the other hand, the subjects trained with a 0.6mA US had a higher performance than the ones trained with a 1.0mA US both when a 3" CS [ $F(1,24)=4.58$ ;  $p=0.043$ ] and a 10" CS [ $F(1,24)=14.24$ ;  $p=0.001$ ] were used.

The LTR session has been subdivided into 3 blocks of 10 trials each. Figures 2 and 3 also show the level of LTR (avoidances and latencies) in each of the blocks for each of the experimental groups. With regard to the number of avoidances, both on the additional learning session and in the extinction session all the groups showed a significant ascending linial evolution throughout the session [ $F(1,24)=40.12$ ;  $p<0.001$  and  $F(1,26)=9.67$ ;  $p=0.005$ , respectively], although the evolution during the additional learning session can also be explained by a second degree function [ $F(1,24)=4.63$ ;  $p=0.042$ ]. More specifically, both in the additional learning session and in the extinction

## RETENTION

### Additional Learning



### Extinction

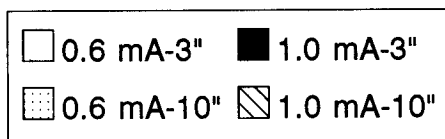
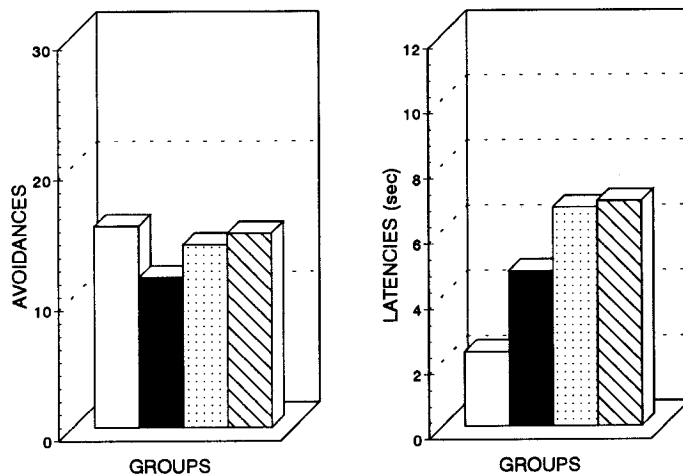
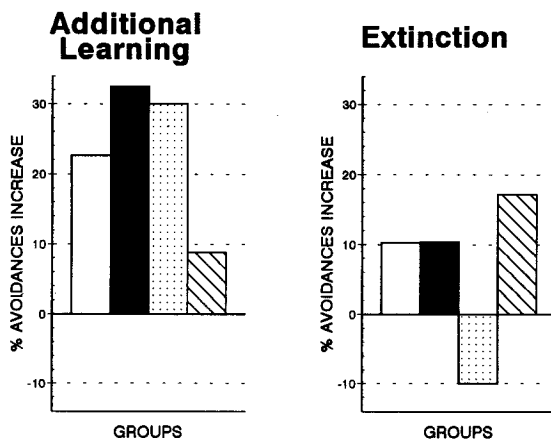


Figure 4: Results corresponding to the two LTR tests: additional learning (figures on the top) and extinction (figures on the bottom) for each experimental group. The left figures show the mean number of avoidance responses, while the right figures show the mean latency of responses in seconds.



## INCREASE OF THE RESPONSE ACQUISITION-RETENTION

### AVOIDANCES



### LATENCIES

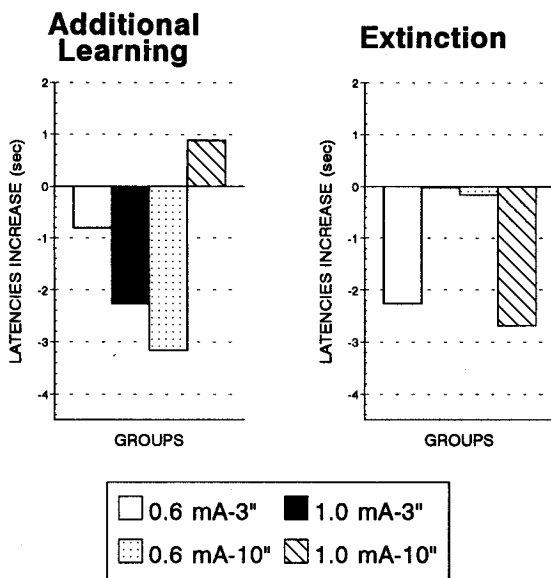


Figure 5: Response increases observed during the two LTR tests compared to the acquisition session, for each experimental group. The figures on the top show the percentage increase of the number of avoidances observed during the additional learning session compared to the acquisition session (left figure) and during the extinction session compared to the acquisition session (right figure). The figures on the bottom show the results corresponding to the changes in the latencies of responses.

session the number of avoidances increased in the second block compared to the first block [ $F(1,24)=23.53$ ;  $p<0.001$  and  $F(1,26)=8.47$ ;  $p=0.007$ , respectively]. The number of avoidances of the groups trained with a 3" CS showed also an increase in the third block of the additional learning session compared to the second block [ $F(1,26)=15.87$ ;  $p<0.001$ ], while the performance of the rest of the groups was maintained. However, this increase was not observed in the extinction session. With regard to the latencies, a descending linear evolution was evidenced throughout the additional learning session [ $F(1,24)=5.5$ ;  $p=0.028$ ], but not during the extinction session.

Another result deserving attention is the percentage of improvement of performance (avoidances increase or latencies decrease) shown by the experimental groups in the LTR with regard to the acquisition session. Those results are depicted in Figure 5. As shown in this figure, all the experimental groups showed a significant increase in the number of avoidances during the additional learning session compared to the acquisition session [ $F(1,24)=29.11$ ;  $p=0.004$ ]. A simple effects analysis indicated that this increase was significant in two groups: the one that had shown the highest level of performance during acquisition [0.6mA-10" group;  $F(1,24)=9.71$ ;  $p=0.005$ ] and the one showing the lower level of performance during acquisition [1.0mA-3" group;  $F(1,24)=17.18$ ;  $p<0.001$ ]. On the contrary, the performance during the extinction session did not show significant differences compared to that in the acquisition session.

The above indicated results were not corroborated by the analysis of the latency of responses, since no significant differences between acquisition and either LTR sessions were found.

### *Survival Analysis*

#### *Acquisition*

To carry out the survival analysis a lear-

ning criterion of 5 consecutive avoidance responses has been chosen, and it has been analyzed whether differences among groups existed regarding the number of trials required to reach the established criterion.

Figure 6 shows the survival function of the acquisition session for the 4 experimental groups. As it can be seen, differences seem to exist among groups regarding the number of trials required for a given proportion of subjects to show the learning criterion. This observation was verified by the Mantel-Cox test, which showed the existence of significant differences among groups [ $S(3)=10.614$ ;  $p=0.014$ ]. Specifically, the animals trained with a 0.6mA US and a 10" CS acquired more rapidly the learning criterion than the subjects trained with a 1.0mA US (regardless of the duration of the CS) [10" CS:  $S(1)=5.904$ ;  $p=0.0151$ ; 3" CS:  $S(1)=7.035$ ;  $p=0.008$ ]. Contrarily, the intensity of the US does not seem to be so significant when the CS lasts 3", since the subjects in 0.6mA-3" group were not significantly different from 1.0mA-3" subjects, but did not differ either from the group reaching the highest level of acquisition (0.6mA-10" group).

On the other hand, there were also differences regarding the percentage of subjects reaching the learning criterion. This percentage was higher in the groups trained with a 0.6mA US (85% in 0.6mA-10" group and 72% in 0.6mA-3" group) than in the groups trained with a 1.0mA US (50% in 1.0mA-10" group and 40% in 1.0mA-3" group).

Another question that can be answered with this kind of analysis is how many trials are needed for each group to reach an asymptotic level. In the acquisition session, it can be seen that, in general terms, when an animal has not reached the learning criterion after 40 trials, it will fail to reach it even if the session is lengthened to 60 trials. From a qualitative point of view, it is remarkable that the only group showing further improvements in the level of learning after trial 40

## SURVIVAL FUNCTION ACQUISITION SESSION

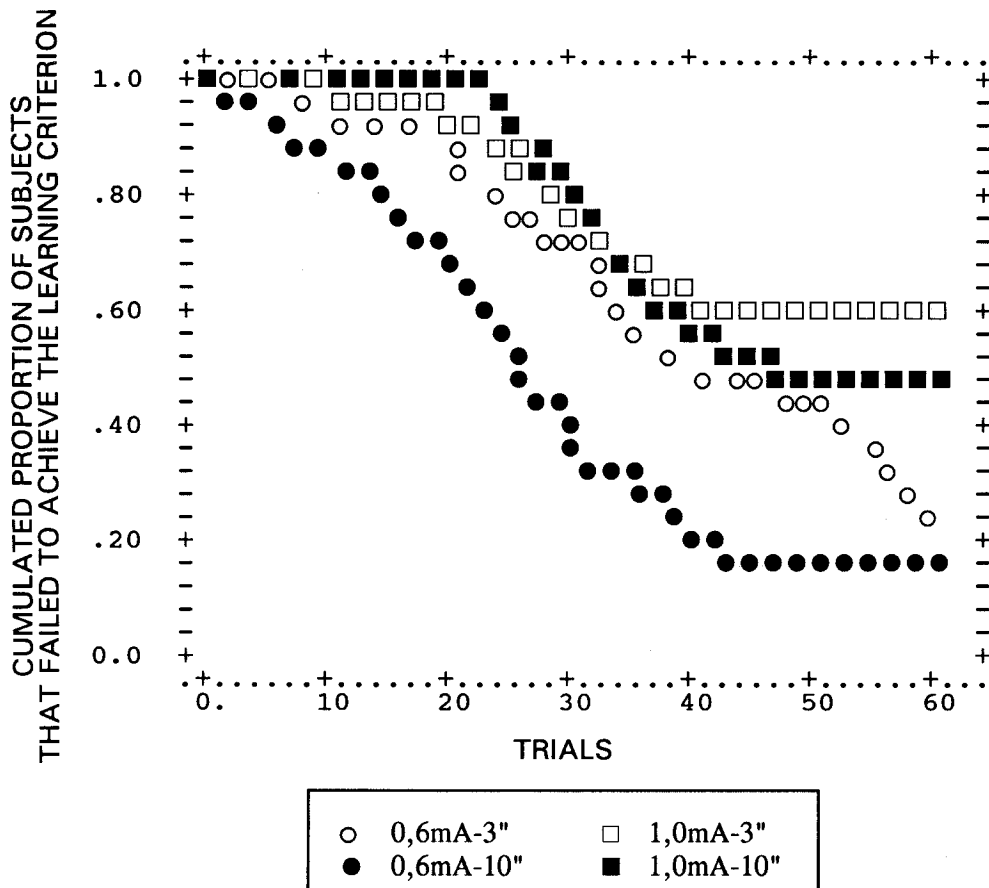


Figura 6: Survival function corresponding to the acquisition session. This figure shows the cumulated proportion, in each trial, of subjects in every experimental group that failed to achieve the learning criterion (5 consecutive avoidance responses). It can also be shown the evolution of conditioning, for each experimental group, throughout the acquisition session.

is the 0.6mA-3" group. The intensity of the US used with this group (0.6mA) seems to be a favourable parameter for a high proportion of subjects to reach the learning criterion (a similar proportion to that shown by the group having an overall better performance during that session, 0.6mA-10" group), but, on the other hand, the duration

of the CS is the least favourable, and therefore more trials are needed to reach a level similar to that of 0.6mA-10" group.

### Retention

During the LTR tests, differences among groups were observed only when an additional learning session was used [S(3)=9.844;

## SURVIVAL FUNCTION ADDITIONAL LEARNING SESSION

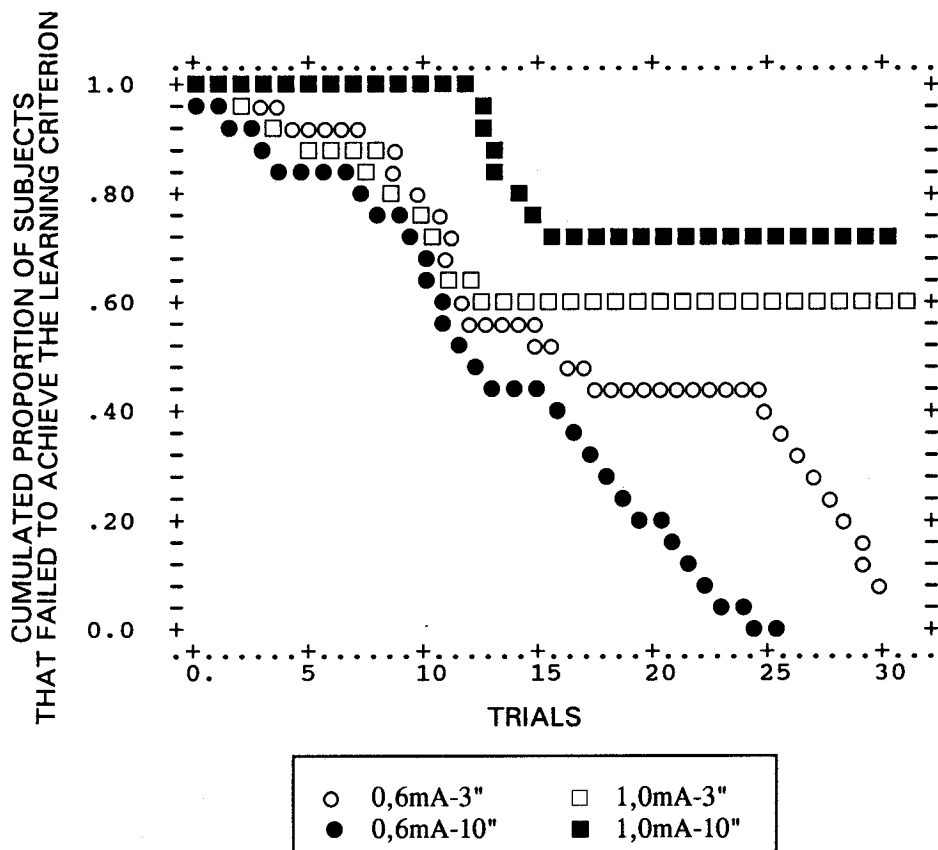


Figura 7: Survival function corresponding to the additional learning session. This figure shows the cumulated proportion, in each trial, of subjects in every experimental group that failed to achieve the learning criterion (5 consecutive avoidance responses). It can also be shown the evolution of conditioning, for each experimental group, throughout this session.

$p=0.019$ ], but not when animals were subjected to an extinction session.

The survival analysis relating to the additional learning session showed that the groups that had shown a better acquisition level were also the ones showing a better performance during this session (see Figure 7). In general, the intensity of the US seems to be a significant factor, since the learning

criterion was more easily reached with a 0.6mA US than with a 1.0mA US. [ $S(3): 4.691; p=0.0303$ ]. Nevertheless, this effect seems to depend upon the CS duration, since it was only significant when the CS lasted 10'' [ $S(1)=7.274; p=0.007$ ], but not when it lasted 3''. It is also remarkable that 0.6mA-3'' group, which during the acquisition session did not differ from any other

group, during this LTR test had a significantly higher performance than 1.0mA-10" group [ $S(1)=3.992$ ;  $p=0.045$ ]. Thus, in general terms the Ss in 0.6mA-3" group required less trials to reach the established criterion than the groups conditioned with 10mA.

During this LTR test, and similarly to what had been observed during acquisition, there were also differences in the percentage

of subjects in each group reaching the learning criterion. A higher percentage of subjects reached the learning criterion in those groups trained with a 0.6mA US (100% in 0.6mA-10" group and 85% in 0.6mA-3" group) than in the groups trained with a 1.0mA US (29% in 1.0mA-10" group and 40% in 1.0mA-3" group). Comparing those percentages with the ones observed during

### SURVIVAL FUNCTION EXTINCTION SESSION

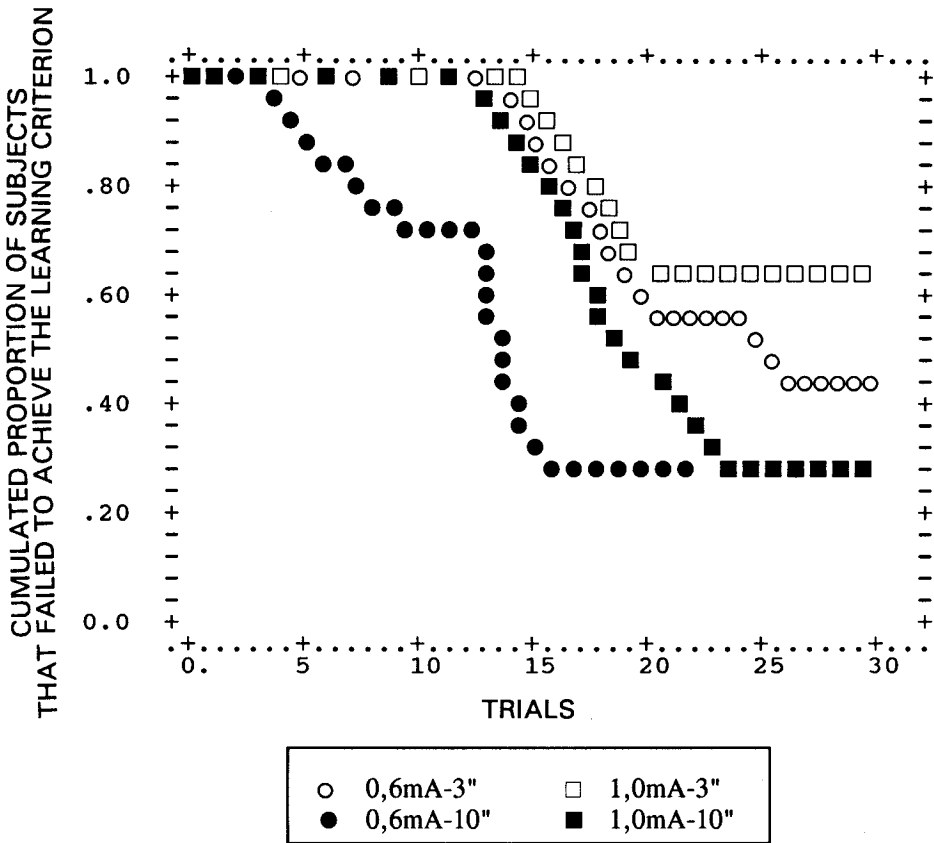


Figure 8: Survival function corresponding to the extinction session. This figure shows the cumulated proportion, in each trial, of subjects in every experimental group that failed to achieve the learning criterion (5 consecutive avoidance responses). It can also be shown the evolution of conditioning, for each experimental group, throughout this session.

the acquisition session, it can be seen that they are increased in the groups trained with a 0.6mA US, while being reduced in the groups trained with a 1.0mA US.

In this sense those groups trained with a 1.0mA US did not show any improvement of performance during the additional learning session compared to the acquisition session. On the other hand, the groups trained with a 0.6mA US showed further improvements during this LTR session.

With regard to the extinction session, and as indicated above, no significant differences among groups were detected on that session (see Figure 8). In spite of that, the contrast analyses between groups evidenced that subjects in 0.6mA-10'' group, which had shown the highest performance both in the acquisition session and in the additional learning session, were also the ones requiring a lower number of trials to reach the learning criterion during the extinction session, specially when compared to the groups trained with a 1.0mA US [10'':  $S(1)=7.274$ ;  $p=0.007$ ; 3'':  $S(3)=3.568$ ;  $p=0.058$ ].

Another remarkable issue to have into account is the fact that the avoidance behavior did not show any evidence of extinction in any of the groups. Furthermore, if we have into account the percentage of subjects reaching the learning criterion during the extinction test (0.6mA-3'': 64%; 0.6mA-10'': 72%; 1.0mA-3'': 38%; 1.0mA-10'': 72%), no inverse relationship between this variable and the acquisition level can be aduced. Nevertheless, the group showing a better acquisition level was the one requiring a lower number of trials (15) to reach an asymptotic level, i.e., to stop improving its performance during the extinction test.

#### *Time-Series Analysis (TSA)*

As indicated in the introduction of this paper, the TSA allows to study the evolution of a given variable over time and to compare this temporal evolution in different groups or in different individual subjects. The first

criterion to apply the TSA is, of course, to have longitudinal data about the variable to be studied. On the other hand, those data have to show serial dependency; i.e. the data obtained on different times have to autocorrelate. Another important criterion to apply this analysis is to have a high number of observations (Box and Jenkins, 1970, recommend a minimum of 50 observations) and that the time-intervals separating the different observations be constant. In our case, and having into account the just-mentioned criteria, we have applied the TSA to the evolution of the latencies of responses over the acquisition session (60 consecutive trials). The first step to do so has been to search for an optimal model that could be adjusted to the evolution of the latencies of responses of each group throughout the session. To that effect, the ARIMA method (SPSS-PC) has been used. Generally, several putative models are tried and one of them is chosen having into account several adjustment indices given by the ARIMA method. The second step consists of estimating (from the parameters of the chosen model) the 95% confidence interval (95CI) of the values indicated by the model. The upper and lower limits of this interval will be considered as the statistical criterion (see Domènech, 1985) to compare the curves corresponding to different groups or to different subjects (see Capdevila, Cruz and Viladrich for a description of the application of TSA to the field of Psychology).

In our case, an ARIMA model has been adjusted to the latencies of responses during the acquisition trials for each of the 4 groups subjected to different conditioning conditions. After that, we have analyzed whether the temporal evolution of the acquisition of conditioning shows differences depending on the intensity of the US.

Table 2 shows the parameters of the ARIMA models fitted to the latencies of each of the 4 experimental groups considered during the acquisition session, as well

Table 2

Parameters of the ARIMA models adjusted to the latencies of response of each experimental group during the acquisition session and values of the fitness statistics for those models.

GROUP	0.6mA-3"	0.6mA-10"	1.0mA-3"	1.0mA-10"
ARIMA MODEL	(1,1,0) AR1=-0.47, p<0.001	(0,1,1)	(0,1,1)	(1,1,0) AR1=-0.41 p<0.001
Degrees of Freedom	59	59	59	59
AIC	222.31	193.83	212.67	210.64
Mean Error	0.023	-0.0006	-0.10	-0.01
Residuals Std. Err.	1.56	1.22	1.41	1.41
Durbin-Watson	2.00	2.05	2.09	2.02

as the values of the adjustment indices for each model.

The top picture in Figure 9 depicts the evolution curve of the mean latencies of subjects in 1.0mA-3" group throughout the acquisition session. Overimposed are also the values of the lower and upper limits of the 95CI predicted by the ARIMA model (1,1,0) adjusted to 0.6mA-3" group. As seen in this figure, the temporal evolution of the latencies of responses is very much similar in both groups, and only in a few time points the values corresponding to the 1.0mA-3" groups do not fit into the 95CI predicted for the 0.6mA-3" group. In conclusion, the temporal evolution of those two groups, although not wholly coincidental, does not show appreciable differences. Therefore, when the CS lasts 3", the intensity of the US does not seem to have any remarkable influence upon the subjects' performance.

Similarly, the bottom picture in Figure 9 compares the 95CI of the ARIMA model (0,1,1) adjusted to the latencies of 0.6mA-10" group to the mean latencies of subjects in group 1.0mA-10". As it can be observed, in a high number of trials the latencies of the latter are situated above the upper limits of the 95CI of the former group; this fact implies that with a 10" CS, the subjects trained

with a 1.0mA US show higher latencies (and, therefore, a worse performance) than those trained with a 0.6mA US. In other words, the TSA indicates (coinciding with the results of the analysis of variance) that the intensity of the US has a significant influence upon the acquisition of conditioning depending on the CS duration, suggesting that this influence is of higher magnitude when the CS lasts 10" than when it lasts 3", and this fact is not reflected significantly by the analysis of variance concerning the acquisition session. The TSA allows also to determine the specific trials when the differences between groups are of higher magnitude. Specifically, it can be observed that in trials 21 to 31, the curve corresponding to 1mA-10" group runs near to the upper limit of the 95CI of 0.6mA-10" group. From trial 36 on, this curve is clearly above the upper limit of this interval in most trials. Therefore, the differences between groups are evidenced mainly during the second part of the acquisition session, specially from trial 36 on.

It is well known that the statistical descriptives (such as group means) are not always representative of some of the subjects belonging to a given group. In that sense, the TSA makes it possible to analyze, based on statistical criteria, whether the actual values

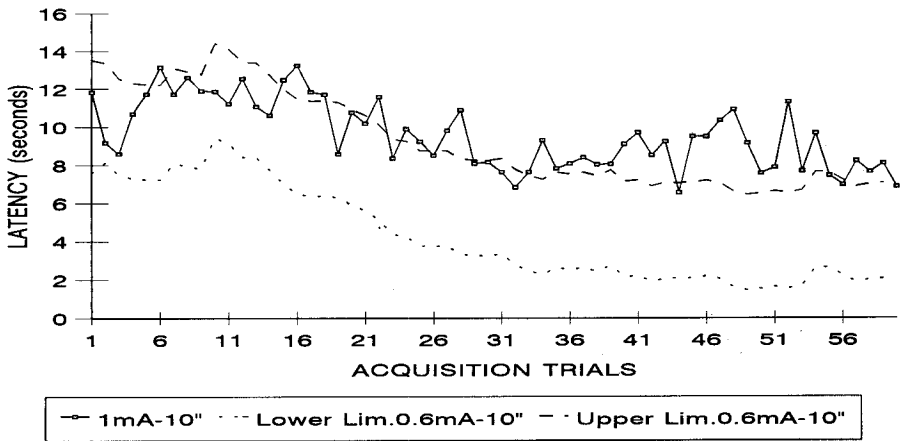
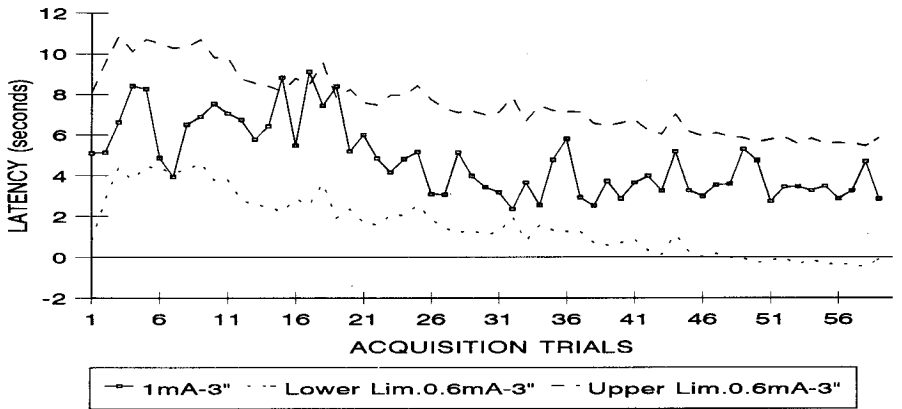


Figure 9: The top figure shows the mean latencies of response of the 1mA-3'' group for each acquisition trial, together with the upper and lower limits of the 95% confidence interval estimated from the ARIMA model (1,1,0) adjusted to the performance of the 0.6 mA-3'' group. The lower figure shows the mean latencies of response of the 1 mA-10'' group for each acquisition trial, together with the upper and lower limits of the 95% confidence interval estimated from the ARIMA model (0,1,1) adjusted to the performance of the 0.6 mA-10'' group.



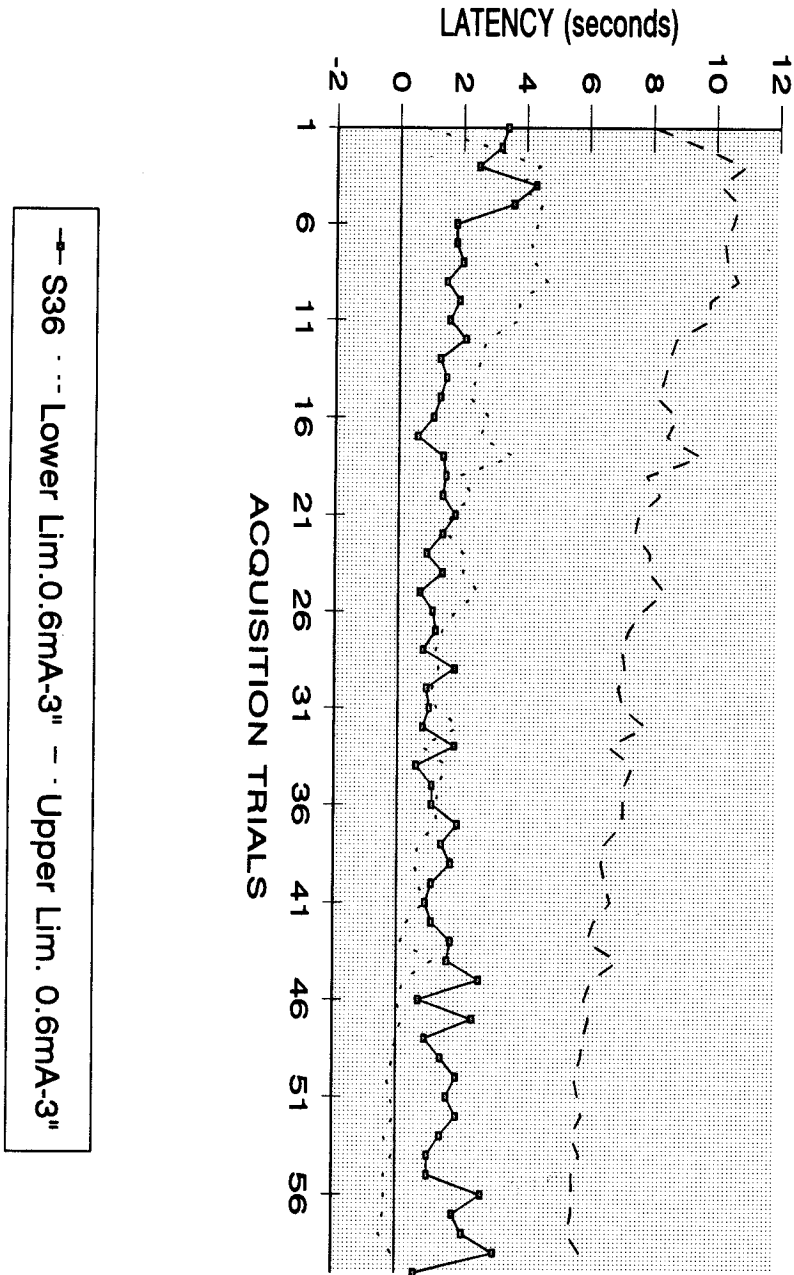


Figure 10: Latencies of responses of subject 36 (belonging to the 0.6mA-3" group) for each trial in the acquisition session and upper and lower limits of the 95% confidence interval corresponding to the ARIMA model (1,1,0) adjusted to the mean latencies of its group.

of a subject fit to a model generated from the mean data of its groups, as well as to compare within-subject data in single case experimental designs (see, for example, Capdevila and Cruz, 1992). To illustrate this utility, Figure 10 depicts the 95CI of the ARIMA model (1,1,0) adjusted to the latencies of 0.6mA-3" group, together with the latency values corresponding to one of the subjects in this group, subject 36. As it can be seen, the performance of this subject differs significantly from the mean performance of its group, specially on the trials of the first part of the training session, and this fact cannot be evidenced by the traditional analysis of variance. Specifically, the latencies of response of this subject are lower than the lower limit of the 95CI estimated for its group at the beginning of the session, although these differences disappear on the last trials.

## DISCUSSION

The results found in the present work show that the conditioning parameter having the most decisive influence upon the level of acquisition achieved by the subjects is the intensity of the US. Under the conditions used by us, the subjects' performance is considerably better with an US of 0.6 mA than with an US of 1.0 mA. On the other hand, and according to the analysis of variance, the duration of the CS does not seem to exert any significant influence upon the acquisition level of the subjects. Nevertheless, this assessment cannot be regarded categorically, since both time series and survival analyses have shown that the superiority of performance with an US of 0.6 mA is much more evident when the CS lasts 10 seconds than when it lasts 3 seconds. In other words, there seems to be an interaction between the CS duration and the US intensity. At all events, we believe that it is of great importance to apply in each case the statistical tests which might be more sensitive to

detect the potential existence of interactions between parameters.

According to several reports about the parameters influencing the rate of acquisition of two-way active avoidance the following outstanding data can be remarked:

a) Most works have indicated the existence of an inverse relationship between the intensity of the US and the number of avoidances (Theios et al., 1966; McAllister et al., 1971; Tobeña, 1979). This seems to be also applicable to our results, specially when a 10-sec CS is used. Nevertheless, Archer et al. (1984) have specified that such an inverse relationship is only observed during the first conditioning sessions, but it can disappear or even be inverted on consecutive sessions. Although in the present work a single training session has been used, it can be said, as shown by time-series analysis, that the inverse relationship between the intensity of the US and performance is specially manifested during the second half of the acquisition session. And, as indicated by the analysis of variance and the survival analysis, this relationship is maintained on LTR. Therefore, in our case such a relationship does not show any sign to disappear with time, although there is no doubt that further conditioning sessions would have to be performed so as to be able to formulate a reliable conclusion about that issue

b) In general terms, the performance of the subjects usually improves when the duration of the CS is increased (Coll, Martí, Portell and Morgado, 1993). Nevertheless, according to Hoffman (1966), the optimal duration of the CS to improve the performance ranges from 5 to 10 seconds, while a shorter CS does not allow that an adequate level of learning be achieved. The results in our work do not completely agree with this assessment, since, as indicated by the survival analysis, when the CS lasts only 3 seconds a relatively high percentage of subjects achieves the learning criterion. This percent-

tage depends on the intensity of the US, ranging from 40%, with a US of 1 mA, to 70% with a US of 0.6 mA.

Regarding the LTR of learning, not many differences have been found between the two different methods used to measure it, additional learning and extinction. In both cases, the subjects' performance during LTR session does not show statistical differences compared to the acquisition session. As shown in several works (Tobeña, 1979; Fernández, 1983), the omission of the US does not seem a sufficient condition to extinguish the two-way active avoidance responses. Only those subjects trained with a 0.6 mA US and a 10-sec CS showed a (non-significant) tendency to make a lower number of avoidances when compared to the acquisition session. Thus, although on the one hand those subjects needed the lowest number of trials to reach the performance criterion on the extinction session, on the other hand they were also the subjects reaching more rapidly an asymptotic level in that session; in other words, they were the first ones to stop improving their performance. They were also, precisely, the subjects that had shown a better acquisition level (see Figure 5) and this might be the reason why they could be aware of the disappearance of the contingency relationship between the CS and the US. Whatever that might be, the two-way active avoidance conditioning responses seem to be specially difficult to eliminate once acquired, and special procedures have had to be designed to reach a certain extinction level of those responses (i.e., ordinary extinction, response prevention or flooding, delay warning signal termination, etc) (Solomon, Kamin and Smith, 1953; Page and Hall, 1953; Katzev, 1967).

The analysis of variance does not make it possible to detect differences between the two methods used to assess LTR. Instead, the survival analysis seems to be sensitive to those differences. Thus, this analysis has clearly indicated that only during the additional

learning session, but not during the extinction session, there were differences among groups. Altogether, it shows that in those groups with a higher percentage of subjects achieving the predetermined performance criterion (5 consecutive avoidance responses), i.e., the groups trained with a 0.6 mA US, this percentage continued increasing during the additional learning session. Contrarily, this percentage was reduced in those groups which had shown a lower percentage of subjects capable of achieving the learning criterion, i.e., in those groups trained with a 1 mA US.

Having into account the results of the analyses which have been carried out, the extinction procedure does not seem to be adequate to assess the level of LTR of two-way active avoidance. It seems that an additional learning session, in which the same contingency relationship between the intervening stimuli than that used during the acquisition session, might be more appropriate to that effect. At all events, the level of LTR might probably be better assessed during the first trials of the additional learning session, thus minimizing the learning effect associated to the additional conditioning trials.

With regard to the second objective of our work (i.e., to illustrate the utility of certain non traditional statistical analyses within the background of psychological studies), it has been shown that, certainly, both time-series analysis and survival analysis, besides reinforcing some conclusions drawn from the analysis of variance, can in several instances disclose some aspects which are not evidenced with the traditional methods. In the present work, time-series analysis has made it possible to evidence the existence, on the acquisition session, of an interaction between the intensity of the US and the CS duration, which was not shown by the analysis of variance. Furthermore, it has given useful and detailed information about the specific trials in the acquisition session when differences existed between the compared groups. On

the other hand, it has made it possible to analyze whether the performance of a given subject fits to the mean performance of its group over all the session. By its turn, the survival analysis, besides allowing to assess the percentage of subjects achieving a predetermined learning criterion, has

afforded valuable information to determine differences between the two methods used to evaluate LTR.

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