Discrimination of acoustic patterns in rats using the water T-maze

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The extraction of abstract rules and their generalization to new items has been proposed to be at the heart of higher cognitive functions such as language. Research with animals has shown that various species can extract rather complex patterns from the input, as well as establish abstract same/different relations. However, much of these findings have been observed after extensive training procedures. Here, we tested rats' capacity to discriminate and generalize tone triplets that entailed a repetition from triplets that followed an ordinal, non-repeating pattern following a relatively short discrimination training procedure in a water T-maze. Our findings demonstrate that, under this procedure and after only 12 sessions, rats can learn to discriminate both patterns when a reliable difference in pitch variations is present across them (Experiment 1). When differences in pitch are eliminated (Experiment 2), no discrimination between patterns is found. Results suggest a procedure based on a water T-maze might be used to explore discrimination of acoustic patterns in rodents.

A key aspect in mastering complex cognitive abilities is to extract abstract relations between specific elements. At a minimum, this entails being able to tell if two items are the same or different (for a recent review, see Wasserman & Young, 2010). There is ample empirical evidence showing that several animal species are able to establish abstract same/different relationships between elements. These include birds, dolphins, and primates (Cook & Brooks, 2009; Mercado, Killebrew, Pack,

Mácha and Herman, 2000; Wasserman, Fagot and Young, 2001; Wright, Shyan and Jitsumori, 1990) and even insects such as bees (Giurfa, Zhang, Jenett, Menzel and Srinivasan, 2001). Apart from humans, other animals are also able to differentiate between more complex abstract acoustic patterns (as in ABA and AAB patterns, where A and B stand for different sounds). More specifically, Murphy, Mondragón and Murphy (2008) provided data suggesting that after training, rats could discriminate sequences of three elements that followed a given pattern (e.g. AAB) from sequences that violated it (e.g. ABA or ABB). Similarly, de la Mora and Toro (2013) showed that with lever-pressing training, rats can learn with the same ease simple acoustic rules implemented over both consonants and vowels. However, these studies relied on extensive discrimination training procedures to obtain successful rule learning performance.

In the present study we wanted to further explore rats' rule learning abilities using a novel discrimination procedure based on a T-shaped water maze. There is a long and productive history of research on comparative cognition using the water maze (e.g. Morris, 1984). This setting has extensively been used to study a wide array of issues in Cognitive Neurosciences including memory decay, visual discrimination, and most notably, spacial navigation (for a review, see Vorhees and Williams, 2006). But, to the best of our knowledge, there are very few studies (if any) taking advantage of this setting for acoustic discriminations. The simplicity of the water maze makes it an interesting tool to explore new applications for studies on learning complex acoustic patterns in which the rat discovers the location of a platform given certain sound cues.

Thus, the aim of the present study is twofold. First, we wanted to explore the discrimination of complex acoustic patterns implemented over musical chords in a water T-maze. Second, we wanted to assess which cues might rats use more easily for performing such discrimination. In two experiments we tested whether rats could learn to discriminate sequences of three piano tones following an abstract pattern containing identity relationships between their elements, from sequences not containing such relationships. Importantly, we also tested if they could generalize such discrimination to new items not presented during training. Thus, the goal of Experiment 1 was to test the discrimination and generalization of two different patterns, one containing an adjacent repetition (AAB; meaning that the first two tones were the same and the third tone was different), and one with no repetitions and a changing melodic contour (composed of three tones with Medium pitch, High pitch, and Low pitch; MHL). In Experiment 2 we decided to test rats' discrimination abilities between AAB and ABC triplets instantiated over monotone syllables. The absence of a pitch contour

in such triplets would allow us to tackle the question of the extent to which rats are mainly relying on pitch changes (and not on the presence of a repetition) to differentiate between triplets in the previous experiment.

EXPERIMENT 1

In this experiment we used a water T-maze to train the rats to discriminate among tone sequences that would follow two different patterns (AAB and MHL). Sequences following an AAB pattern would signal the location of an escape platform would be on one side of the T-maze, while sequences following a MHL pattern would signal the location of the platform would be on the opposite side.


METHOD

Subjects. The subjects were 12 Long-Evans male rats of 3 months of age. They were caged in groups of two, with water and food ad libitum. All experimental procedures were approved by the Comité de Ética de Experimentación Animal from the Universitat de Barcelona, and complied with guidelines from the Catalan and Spanish governments for the treatment of laboratory animals.

Apparatus. We used a circular pool of 100 cm in diameter and 40 cm deep filled with water up to 11cm. The water was colored with latex, so it was not transparent. Using brown Plexiglas planks, a T-shaped maze was built. The upper side of the maze was 100 cm long, while the central part was 64 cm long (see Fig. 1). A white circular (10 cm in diameter, 10 cm high) platform was hidden at the end of either arm of the T-shaped maze. Since the water was colored, the platform was not visible. A black curtain surrounded the pool to prevent rats from focusing on salient visual landmarks (such as the door of the room, the computer, etc). A Pioneer Stereo Amplifier A-445 and one E. V. (S-40) speaker located in front of the pool (facing the position where animals started each trial) were used to present the stimuli.

Stimuli. The stimuli were triplets made out of ten piano tones (six tones were used during training, and four were used for the generalization test). The tones were computer-generated as described in Endress, Dehaene-Lambertz and Mehler (2007). Sampling rate was set to 16 kHz. Fundamental frequency of the tones increased as multiples in Lydian mode.

The tones used for training had frequencies of 440, 493.88, 587.33, 698.45, 880, and 1046.5 Hz (equivalent to notes A4, B4, D5, F5, A5, and C6). Fundamental frequency of the tones used for generalization test was 523.25, 659.25, 783.99, and 987.76 Hz (equivalent to the notes C5, E5, G5, and B5). Duration of each tone was set to 330 ms, and each tone had a falling contour starting around 100 ms. In this way, each tone was easily perceived as independent, and could not be confused with following tones.
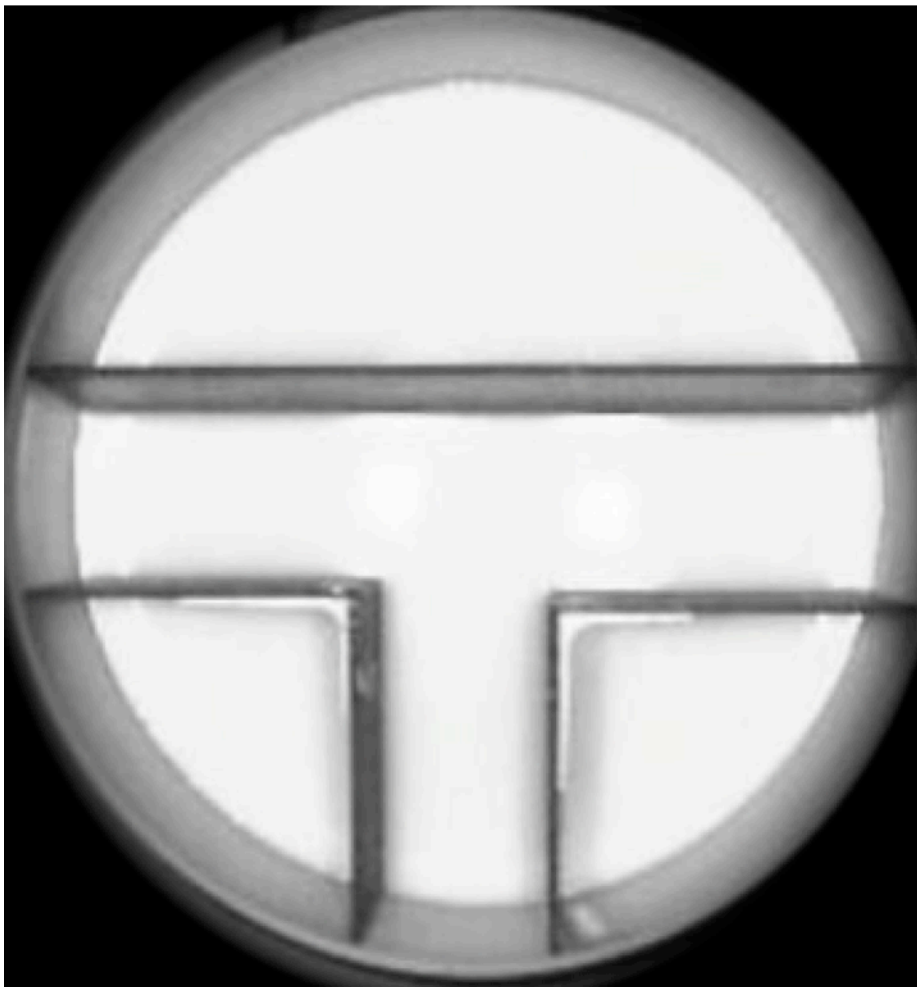
Figure 1. T-shaped water maze used during the present study. A circular platform was hidden on an arm of the maze and acoustic stimuli predicted its location.

Tones were combined by three to form 20 triplets with no silences between tones in each triplet. Previous experiments have shown that rats easily perceive and discriminate tones changing in pitch presented within this frequency range (de la Mora, Nespor and Toro, 2013). Half of the triplets followed an adjacent repetition pattern (AAB), and the other half followed an ordinal pattern (MHL). An AAB pattern meant that the first two tones were identical and the third one was different (e.g. High-High-Low or Low-Low-High), yielding sequences such as D5-D5-A4 or B4-B4-F5. A MHL pattern referred to a sequence of three different tones, having the second one a higher pitch than the first, and the third tone a lower pitch than the previous two (e.g. Middle-High-Low), yielding sequences such as D5-A5-B4 or B4-F5-A4. We choose to compare these two patterns because it has been shown repetition-based patterns might provide the basis for learning more complex structures (Marcus, Vijayan, Bandi Rao and Vishton, 1999), and are easily processed by both human adults and infants (Toro, Sinnett and Soto-Faraco, 2011). Stimuli were presented at 70 dB.

Procedure. The experiment consisted of a training phase and a test phase. The day before starting the discrimination training procedure in the T-shaped water maze, we ran a habituation session so rats would familiarize with the apparatus and procedure. This gave them the opportunity to learn that a platform was placed at the end of either arm of the maze. During this session, rats were placed individually at the base of the T-maze and let to freely swim during one minute. If a rat found the platform during this time, the animal was left on it for 20 seconds. No auditory stimuli were presented during this habituation session.

During the training phase there were 12 training sessions, one session per day. Each session consisted in eight trials. In each trial, rats were placed individually at the base of the "T" and let to freely swim. Tone triplets of a given pattern (repetition-based patterns or ordinal patterns) were played during the entire trial with a 1-second silence between each triplet. Repetition-based triplets were played during half of the trials, and ordinal triplets were played during the other half. The order of pattern presentation was balanced within each session and across training sessions. Importantly, the position of the platform depended on the pattern of the triplets being played (i.e. it was located in the right arm of the maze when repetition-based triplets were played, while it was located in the left arm of the maze when ordinal triplets were played). Thus, the pattern of triplets being played was a perfect predictor of location of the platform, which was balanced across rats. For half of the rats (n=6) a given pattern (e.g. AAB) signaled the

platform was on the right arm of the maze, while for the other half it signaled it was on the opposite arm. If the rat's first choice was to swim into the arm of the maze where the platform was located, the trial was coded as "correct". Once the rat found the platform, it was left to rest on it during 20 seconds. On the contrary, if the rat's first choice was to swim into the arm of the maze where there was no platform, the trial was coded as "incorrect". During incorrect trials, the rat was blocked in the arm of the maze during 15 seconds.

The test phase consisted in one session. It was identical in all respects to any training session with the exception that new triplets (made out of new tones) were presented. These triplets followed the exact same patterns as in the training phase. The test phase would allow us to confirm that rats did not learn specific instances, but a more general rule underlying the patterns' arrangement. If so, we expected to find similar responses to the stimuli during the test phase than during the training phase.

RESULTS

Correct and incorrect responses were recorded during training and test phases. To analyze the performance during the training phase (the number of correct responses in each training session), we run a repeated-measures ANOVA with session (1 to 12) and stimuli (AAB and MHL) as within subjects factors. For the test phase, we compared performance across stimuli and against chance. The analysis of the training phase yielded a significant difference between sessions ($F(11, 121) = 2.691$, $p < 0.005$), and stimuli ($F(1, 11) = 7.093$, $p < 0.05$). However, the interaction between both main factors did not reached statistical significance ($F(11, 121) = 1.637$, $p = 0.097$; see Fig. 2), very likely due to the very large variability produced by the present procedure. However, data suggests that through training, rats began to discriminate between AAB and MHL auditory patterns. Similarly, the analysis of the responses during the test phase showed that rats responded differently to AAB and MHL stimuli ($t(11) = -2.284$, $p < 0.05$; see Fig. 3). Correct responses to AAB stimuli actually fell below chance levels (M=25% SEM=9.73; $t(11) = -2.569$, $p < 0.05$). On the contrary, responses to MHL test stimuli were numerically above chance, and this difference was close to statistical significance (M=72.91%, SEM=11.70; $t(11) = 1.959$, $p = 0.076$). So, during this test session, location of the hidden platform was more effective with MHL triplets than with AAB triplets, even though these structures were implemented over new tones not presented during training.
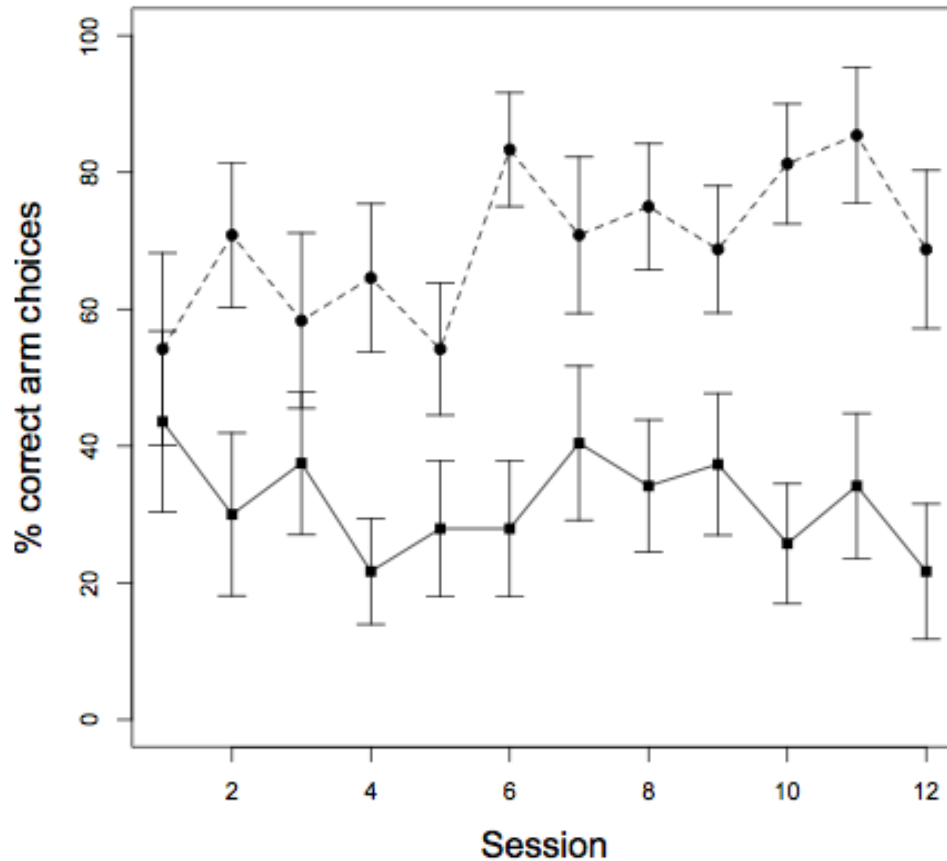
Figure 2. Mean percentage of correct arm choices (and standard error bars) across discrimination training sessions in Experiment 1. The continuous line corresponds to arm choices after AAB sequences. Dotted line corresponds to arm choices after MHL sequences.

These results suggest rats were relying on the melodic contours of MHL triplets (rather than on the repetitions present in AAB triplets) as a salient cue to find the hidden platform. Thus, to further evaluate the contribution of melodic contours on pattern discrimination, we ran a second experiment in which we implemented both types of triplets over monotone syllables. If rats relied only on the melodic contour to discriminate between patterns in Experiment 1, we do not expect to find evidence of discrimination between monotone words or of their generalization in Experiment 2.
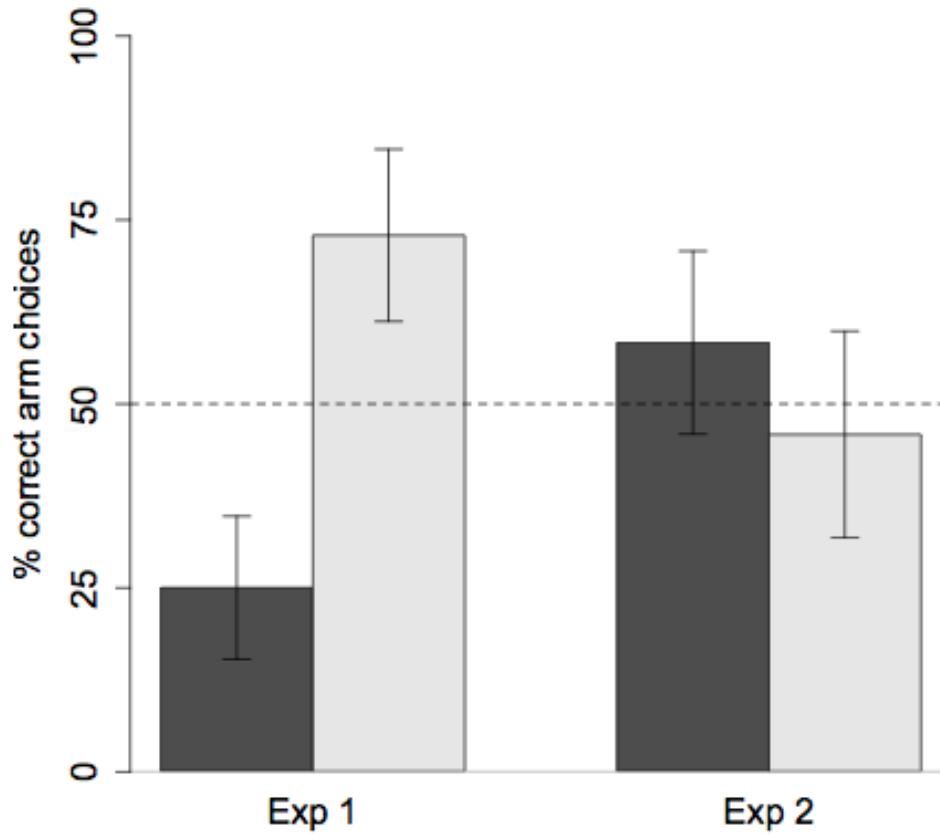
Figure 3. Mean percentage of correct responses (and standard error bars) after repetitive (AAB) and non-repetitive (MHL and ABC) stimuli in Experiments 1 and 2. Dark bars correspond to stimuli with repetitions. Light bars correspond to stimuli with no repetitions. Chance level is marked by line at 50%. Only differences in Experiment 1 reached significance.

EXPERIMENT 2

In this experiment we used a water T-maze to train rats to discriminate among monotone syllable sequences following two different patterns (AAB and ABC). As in Experiment 1, each pattern signaled the location of the escape platform on a different side of the maze.

METHOD

Subjects. Subjects were 12 new Long-Evans male rats of 3 months of age. They were caged in groups of two, with water and food ad libitum.

Apparatus and procedure. The apparatus and the procedure were the same as in Experiment 1.

Stimuli. Forty trisyllabic CVCVCV (C=consonant, V=vowel) nonsense words were synthesized using text-to-speech MBROLA software (available at http://tcts.fpms.ac.be/synthesis) with a female diphone database (it4). Sampling rate was set to 16 KHz, and pitch of all phonemes to 240 Hz. Each word lasted 900 ms, and there were no changes of pitch between syllables. AAB-words had the same syllable in the first and second positions, and a different syllable in the third (e.g *bibigu*, *totora*). ABC-words had three different syllables in each position (e.g. *bimegu*, *kabira*). Half of the forty nonsense words corresponded to AAB-words and the other half to ABC-words. Out of the twenty words following each pattern, sixteen were used during the training phase and four new words, composed by syllables not heard during the training, were used during the test phase. Stimuli were presented at 70 dB.

RESULTS

As in Experiment 1, in Experiment 2 we recorded correct and incorrect responses during training and test phases. A repeated-measures ANOVA of the training phase (the number of correct responses in each training session), with session (1 to 12) and stimuli (AAB-words and ABC-words) as within subjects factors, yielded a non-significant difference between sessions ($F(11, 121) = 0.747$, $p = 0.691$), stimuli ($F(1, 11) = 0.001$, $p = 0.977$), nor a significant interaction between both main factors ($F(11, 121) = 1.366$, $p = 0.197$; see Fig. 4). The analysis of the test phase showed that neither the performance in response to AAB-words (M=58.33% SEM=12.44; $t(11) = 0.670$, $p = 0.517$), nor the performance in response to ABC-words (M=45.83%, SEM=14.05; $t(11) = -0.297$, $p = 0.772$) were above chance. The comparison of the responses to both stimuli was non-significant ($t(11) = 0.510$, $p = 0.620$). Thus, these results suggest that rats did not discriminate between monotone words based in the presence or absence of a repetition.
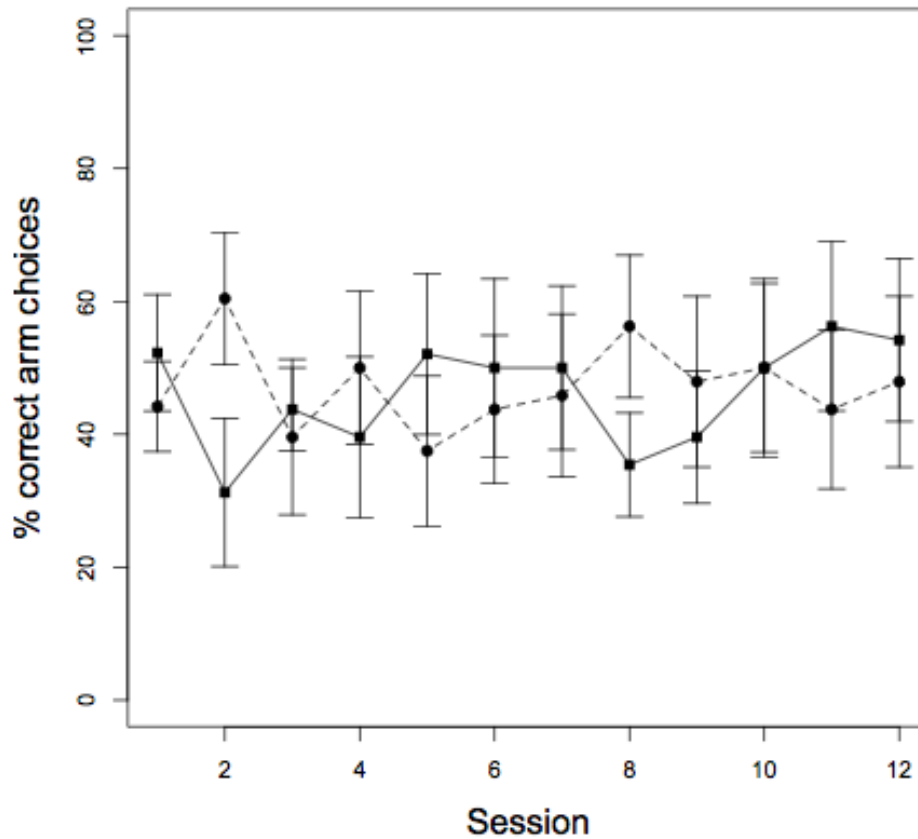
Figure 4. Mean percentage of correct arm choices (and standard error bars) across discrimination training sessions in Experiment 2. The continuous line corresponds to arm choices after AAB sequences. Dotted line corresponds to arm choices after ABC sequences.

DISCUSSION

In the present study we explored the reliability of a water T-maze procedure to test rats' ability to extract and generalize abstract rules implemented over piano tones and syllables. Results showed rats began to discriminate between piano triplets structured over an AAB or a MHL pattern, but not over monotone syllables. The experimental procedure was identical in both experiments, and the stimuli followed very similar structures; both pitted sequences containing a repetition in their first two elements (AAB), against sequences having three distinct elements (MHL

and ABC). The main difference across experiments was that tone sequences in Experiment 1 had a differentiating melodic contour that was absent in monotone syllable sequences in Experiment 2. Results suggest that, everything else being equal, melodic contours help animals in the discrimination and generalization of acoustic patterns.

Previous research has demonstrated rule learning in nonhuman animals. Murphy and colleagues (2008) tested rule learning in rats after pure-tones sequences that followed an ABA pattern using training procedures. Similarly, rule learning over phonological segments has been observed in rats using extensive lever-pressing procedures (de la Mora & Toro, 2013). Using a different species (monkeys; *Macaca mulatta*), Hauser and Glynn (2009) observed rule learning over strings of species-specific vocalizations using a familiarization procedure. More recently, Comins and Gentner (2013) showed that starlings could only generalize acoustic patterns if they were able to classify together the elements that instantiate them. Our results add to this set of studies in two ways. First, they suggest rodents could learn to discriminate acoustic patterns using a relatively short discrimination training procedure (based on the water T-maze). Second, our results suggest rats are learning to discriminate the present tone patterns focusing on differences in melodic contours and not in the presence or location of a given repetition.

Contrary to previous studies with animals (de la Mora & Toro, 2013; Hauser et al. 2009; Murphy et al. 2008), we did not observe generalization of responses to repetitive patterns. This is likely due to differences in experimental methodology. Previous studies in the way rats extract structures over acoustic stimuli (e.g. Murphy et al. 2008; Toro & Trobalón, 2005) have shown that a difference between procedures can yield dissimilar results. For example, using a familiarization procedure, Toro et al. (2005) did not observe any rule learning in rats. However, using a discriminations training procedure, Murphy and collaborators (2008) observed reliable rule learning. In their study, food delivery during training was paired with two sequences of pure tones (i.e. ABA and BAB) and training lasted 75 sessions. The authors found significantly faster response times to novel stimuli following the reinforced rule from those following the non-reinforced rule. Thus, the fact that we did not observe rule generalization over piano triplets using a water T-maze does not preclude the possibility that they might be observed using more stringent training procedures.

Although the results from the present experiments are not conclusive (as some of the target differences only approached statistical significance), they suggest that the use of a water T-maze to explore discrimination of

acoustic patterns might be promising. In Experiment 1 rats began to discriminate between stimuli after only 12 sessions, each session lasting no more than 2 hours. We ignore if longer training periods could have resulted in improved discrimination performance in both experiments. As we mentioned earlier, the present procedure yields high variability that would likely decrease with increasingly longer training. More importantly, the present procedure was quite demanding in terms of cognitive resources. Kovacs and Mehler (2009) have shown that human infants have difficulties in learning two simultaneous rules similar to the ones used in the present study. By designing the study in such a way that animals had to learn two different rules (AAB and MHL in Experiment 1; AAB and ABC in Experiment 2), we might have put them in a non-optimal learning situation. It is thus an open question whether procedural modifications could yield more reliable discrimination results of abstract patterns in rats if they are required to learn only one rule (and not two simultaneous ones). Nevertheless, using a water T-maze could be considered as an attractive alternative to experimental designs relying on lever pressing responses. The water T-maze takes advantage of the natural response of rodents to look for an escape when placed in water. Thus, it avoids lengthy training protocols needed to teach rodents to press a lever to obtain food that are necessary in other procedures. However, future studies using a discrimination procedure based on a water T-maze should take necessary measures aimed at reducing the observed variability. These could include a longer familiarization period before the actual experiment begins (so the animals get more used to the water maze), and more training sessions that might help animals to reach stable patterns of discrimination performance.

Finally, the present results suggest animals found it easier to focus on pitch variations that were especially salient in MHL sequences, rather than on structural repetitions, to find the hidden platform. This contrasts with the salient role observed for repetitions in human adults (e.g. Endress et al. 2007). Similarly, recent experiments show that rodents are more inclined than humans to discover certain patterns over consonants (de la Mora & Toro, 2013). Putatively, this is because humans (and not other animals) tend to assign consonants a lexical role in language processing that makes it difficult to extract abstract patterns over them.   These kinds of contrasts across species could help us to elucidate the extent to which specialized learning modules might be at play in different species while extracting structures from the input (e.g. Gallistel, 2000). Differences across species in the type of constraints guiding learning mechanisms could provide valuable insights about possible cognitive divergences in their evolution.

RESUMEN

Discriminación de patrones acústicos en ratas utilizando el laberinto acuático en T. Se ha propuesto que la extracción de reglas abstractas y su generalización a nuevas estancias es la base de funciones cognitivas superiores, tal como el lenguaje. La investigación con animales ha demostrado que diversas especies pueden extraer y generalizar patrones simples, así como establecer relaciones abstractas de igual-diferente. Sin embargo, tales hallazgos se han observado tras procedimientos de entrenamiento prolongados. Mediante un entrenamiento relativamente breve utilizando un laberinto acuático en T, probamos la capacidad de las ratas para discriminar y generalizar tripletes de tonos que implicaban una repetición, de tripletes que seguían una secuencia ordinal y no repetitiva. Nuestros resultados demuestran que bajo este procedimiento y tras sólo 12 sesiones, las ratas discriminan ambos patrones cuando entre ellos existe una diferencia evidente en variaciones de frecuencia (Experimento 1). Cuando tal diferencia se elimina (Experimento 2), no se observa la discriminación. Los resultados sugieren que un procedimiento basado en un laberinto acuático en T puede ser usado para explorar la discriminación de patrones acústicos en roedores.

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