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Supplementary Materials for

Number-space mapping in the newborn chick resembles humans' mental number line

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This PDF file includes:

Materials and methods

Subjects

Subjects were sixty-four “Hybro” domestic chicks (*Gallus gallus*), a local variety of the White Leghorn breed (*n* = 15 subjects in Experiment 1, *n* = 12 in Experiment 2, and *n* = 37 in Experiment 3). Chicks were obtained weekly, when they were a few hours old, from a local commercial hatchery (Agricola Berica, Montegalda, Vicenza, Italy).

On arrival, the chicks were housed in standard metal cages (28 cm × 32 cm × 40 cm). The rearing room was constantly monitored for temperature (28-31°C) and humidity (68%) and was illuminated by fluorescent lamps (36 W) located 45 cm above each cage. Water and food, placed in transparent glass jars (5 cm in diameter, 5 cm high), were available ad libitum. Twice a day chicks were also accustomed to eat some mealworms (*Tenebrio molitor* larvae) as these were used as reinforcement during training. Chicks were reared in these conditions from the morning of the first (11 a.m.) to the morning of the third day (8 a.m.), when the food jars were removed from the home cages (water was left available), and after a couple of hours (10 a.m.), chicks underwent training. At the end of training each chick was placed back in the home cage and, two hours later, it underwent Test 1. At the end of Test 1, the chick was placed back in the home cage until Test 2, which took place one hour after the end of Test 1. Immediately after the end of all of the behavioral observations, all chicks were caged in social groups of 3-5 birds, with food and water available ad libitum and a few hours later they were donated to local farmers.

Apparatus

The experimental apparatus used during training and tests was located in a separate room adjacent to the rearing room. In the experimental room, temperature and humidity were controlled (respectively at 25°C and 70%) and the lighting was provided by four 58-W lamps (placed on the ceiling, 194 cm above the floor of the experimental apparatus).

The experimental apparatus consisted of a diamond-shaped arena (see Fig. S1) made of uniformly white plastic panels. The external wall consisted of a 20 cm high plastic panel, the floor consisted of a white plastic sheet.

A 'starting' area could be delimited by a transparent removable partition (10 cm × 20 cm) positioned at about 10 cm from the main vertex of the arena. The transparent partition was used to confine subjects for a few seconds before the beginning of each trial (at training as well as at test), in order to give them the possibility of seeing the inner apparatus and the stimuli before being released in the arena.

On each trial chicks were allowed to perform only a single choice. As soon as a chick circumnavigated either screen, it was moved to a separate opaque box (20 cm × 40 cm × 40 cm), to prevent it from seeing the experimenter, while changing the training or test stimuli.

Depending on the experimental phase, we used one (at training) or two identical (at test) white plastic panels (16 cm × 8 cm) located 40 cm away from the transparent partition (hence 50 cm away from the main vertex). Panels were provided with 3 cm sides bent back to prevent the chicks from spotting behind the panel (where the mealworm was hidden during training) before having walked around of it. At training the panel was positioned in the center of the arena, directly facing the main vertex, while during testing the two panels were located symmetrically, one on the right and one on the left with respect to the main vertex, spaced about 30 cm apart.

Stimuli

Training and test stimuli consisted of static 2D images. All stimuli contained a well-defined number of elements, printed on identical white rectangular boards (11.5 cm × 9 cm), which were positioned on the panels in the arena. In each experiment, 20 different training stimuli and a minimum of 5 different test stimuli were used. For each test stimulus we printed two identical copies.

Experiment 1: Training stimuli depicted 5 elements, which consisted of red squares (1.2 cm × 1.2 cm). To prevent the chicks from learning to identify the stimuli on the basis of the spatial disposition of the elements on the boards, for each of the 20 different training stimuli the position of the elements on the board was randomly determined so that the distance between elements varied from 0.3 cm to 3.8 cm. Test stimuli consisted either of a smaller number of elements (2, for the small number test) or a larger number of elements (8, for the large number test, with respect to the training stimuli). Five different test stimuli, differing from one another in the spatial arrangement of the elements, were produced both for the small and for the large number. All of the 15 subjects of Experiment 1 underwent the two tests but in different order. Eight subjects first underwent the small number test, while the other seven first underwent the large number test.

Experiment 2: Training stimuli depicted 20 elements which consisted of red squares (0.6 cm × 0.6 cm). As for Experiment 1, we employed different stimuli (for the spatial disposition of the elements) on each trial, both at training (20 different), and for the tests (5 different for each test). Test stimuli depicted a smaller number of elements (8 for the small number test) or a larger number of elements (32 for the large number test). All of the 12 subjects of Experiment 2 underwent the two tests but in different order. Six subjects first underwent the small number test, while the other six first underwent the large number test.

Experiment 3, Condition 1. Training stimuli depicted 20 elements. The elements used were randomly selected from sets of patterns of different shapes (10 different), colors (10 different) and sizes (10 different, ranging between 0.3 cm and 3 cm). We created twenty different training stimuli (for shape, color, size and the spatial position of the elements). Different stimuli (for shape, color, size and the spatial position of the elements) were used also for each of the test trials. Test stimuli depicted a smaller number of elements (8, for the small number test) or a larger number of elements (32, for the large number test). All of the 12 subjects of Experiment 3 underwent the two tests but in different order. Seven subjects first underwent the small number test, while the other five first underwent the large number test.

Experiment 3, Condition 2. We controlled for the overall area of the elements (i.e., summation of the areas of all elements depicted in each stimulus). In all the experimental phases (training, small number test and large number test), the elements consisted in identical red squares. The dimension of the elements changed relatively to the experimental phase. The training stimuli depicted 20 identical red squares measuring 0.5 cm × 0.5 cm; in this way the area of a single element was 0.25 cm^2 and the overall area of the 20 squares was 5 cm^2. In the small number test, the dimension of the squares was 0.79 cm × 0.79 cm; therefore, the area of a single square was 0.62 cm^2 and the area of the 8 elements was 4.99 cm^2. In the large number test the dimension of the squares was 0.39 cm × 0.39 cm; therefore, the area of a single square was 0.15 cm^2 and the area of the 32 elements was 4.8 cm^2. Moreover, we controlled for the possible use of the overall space occupied by each group of elements, inscribing all elements (either 20, 8 or 32) in a virtual spatial frame measuring either: (8 cm × 10 cm; 7 cm × 10 cm, 5 cm × 9 cm, 6 cm × 6 cm, or 5 cm × 10 cm). We used the same spatial frame for 4 (out of 20) training stimuli, differing one another for the spatial disposition of the elements. On each test we used five testing stimuli. Each of them was inscribed in a virtual spatial frame, which was delimited by four elements positioned on its four vertices.

Experiment 3, Condition 3. We controlled for the possible effect of the overall perimeter (i.e., summation of the perimeters of all elements depicted in each stimulus) and the mean distance among the elements. Moreover, we controlled for the possible effect of the overall space occupied by the group of elements by using the same virtual spatial frames as those in Experiment 3, condition 2.

The dimension of the training elements was identical to that used in Experiment 3, condition 2: 0.5 cm × 0.5 cm. The overall perimeter of the 20 elements was therefore 40 cm. In the small number test elements were 1.25 cm × 1.25 cm squares. Therefore, the overall perimeter of the 8 elements was 40 cm. In the large number test the elements were 0.31 cm × 0.31 cm squares. Therefore, the overall perimeter of the 32 elements was 40 cm. Note the negative correlation between overall area and number in these stimuli. The overall area of the 8 elements (12.5 cm^2) was larger than that of the 32 elements (3.25 cm^2). If the overall area, in the presence of the same perimeter, were the crucial factor underlying space-number mapping, chick would have chosen the panel on the right in the small number test, and that on the left in the large number test.

Moreover, we controlled for the possible effect of the mean distances between the elements in the test stimuli. Density was defined as the mean distance between the elements, and it was computed using a geographic information system (G.I.S.): a computer system used to visualize, analyze, and interpret data to understand relationships, patterns, and trends. We calculated the mean distances in each group of testing stimuli in two ways: a) by considering the distance from the center of each square to the center of all other squares within the same stimulus (mean center distance); b) by considering the distance between the edge of each element and the nearest edge of each other elements (mean edge distance). Both the mean center distance and the mean edge distance did not differ significantly between small and large numbers: mean center distance: 8vs.8, mean rank = 7; 32vs.32, mean rank = 5;

U = 11, P = 0.12. Mean edge distance: 8vs.8, mean rank = 26; 32vs.32, mean rank = 29; U = 11, P = 0.75.

Training

On the morning of the third day (i.e., the testing day) each chick was acquainted with feeding in the arena. A single panel was in place, and a mealworm was located between the starting area and the panel. The chick was at first placed within the arena, in the starting area, for a couple of minutes, free to move around and to get accustomed with the novel environment (no partition was used to confine the bird in this experimental phase). Five mealworms (either small larvae or mealworm pieces were used) were offered to the subject, in separate subsequent trials, and every time the mealworm was positioned closer to the panel. At the end of this phase the chick confidently moved from the starting position and towards the mealworm to eat it.

Following this familiarization phase, the subject underwent a procedure aimed at training the chick to search for food behind the panel. We know from previous studies, in which we used a procedure similar to this, that after having found the food behind a panel depicting a certain number stimulus for a few times, the chick learns to identify the panel by the number depicted on it, in order to look for food (*29*, *30*). Here the chick was confined within the starting area, and a piece of mealworm was positioned in view in front of the panel and was progressively moved (it was tied by invisible thread and slowly dragged by the experimenter) behind the panel. Once released in the arena the chick had to go behind the panel to retrieve the mealworm. The training was over once the chick had gone directly behind the panel and had reached the food reward on 20 consecutive trials. In all training trials chicks received reinforcement. Overall, depending on the chick's behavior, the training phase lasted from 10 to 20 min. Chicks that showed little interest in the food reinforcement (i.e., poor mealworm-following behavior) in

this phase, were discarded from the study: this occurred in about 25% of cases; these chicks are not included in the final sample. All chicks that completed the training phase moved on to the test session.

Testing

This phase comprised two tests (a small number test and a large number test), each consisted of 5 trials. Test trials were never reinforced (i.e., chicks did not receive any food during the tests). At the beginning of each test trial, the chick was confined for about 3 seconds to the starting area, behind the transparent partition, from where it could see the two panels positioned one on its left side and the other on its right side. Then the transparent partition was removed and the chick was left free to move around within the arena. Only the choice for the first panel visited was scored. At that point the trial was ended. A choice was defined as when the head and at least ¾ of the chick's body had entered the area behind one of the two panels (beyond the side edges). At the end of each trial, the experimenter changed the panels; in this period (about 15 seconds were necessary for the experimenter to set up the apparatus for the next trial) the chick was placed in the opaque box outside of the apparatus. As soon as the new pair of stimuli was in place, the chick was moved back to the starting area and remained confined behind the transparent partition for another 3 seconds, after which the transparent partition was removed and the new trial began. This procedure was carried out such that each chick underwent two complete testing sessions of 5 valid trials each.

The chicks' behavior was observed by a monitor connected to a video camera so as not to disturb the chicks by direct observation. All trials were video-recorded. Chicks' behavior was later scored offline by a second experimenter who was unaware of the purposes of the study.

On each test, the number of trials for which each chick chose the left panel was calculated, and the percentages were computed as: (number of left choices / 5) × 100.

Ethics Statement

The experiments complied with all applicable national and European laws concerning the use of animals in research and were approved by the Italian Ministry of Health (permit number: 32662 granted on 19/07/2011). All procedures employed in the experiments included in this study were examined and approved by the Ethical Committee of the University of Padova (Comitato Etico di Ateneo per la Sperimentazione Animale – C.E.A.S.A.) as well as by the Italian National Institute of Health (N.I.H.).

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Fig. S1. Experimental apparatus. Schematic representation of the apparatus employed at training and at test. One panel is represented in the central position, as was in the training set up. The removable transparent partition delimiting the subject starting area by the main vertex of the arena is in place.

Fig. S2. Results of all the Experiments. Percentage choice (means, SE) displayed at test by chicks. The dotted line (y = 50) represents chance level. The asterisks represent a choice significantly different ($P < 0.05$) from chance level.

Fig. S1

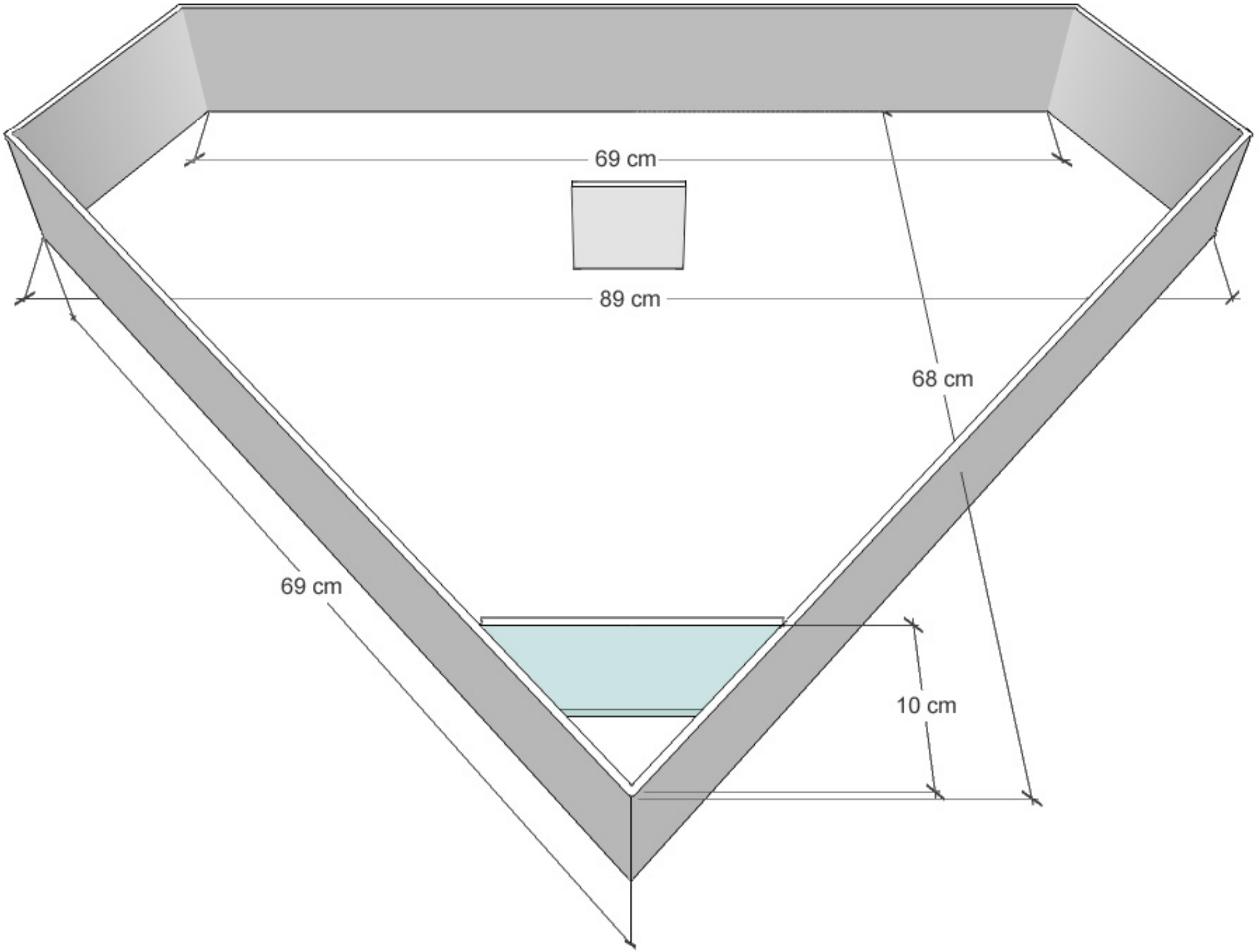
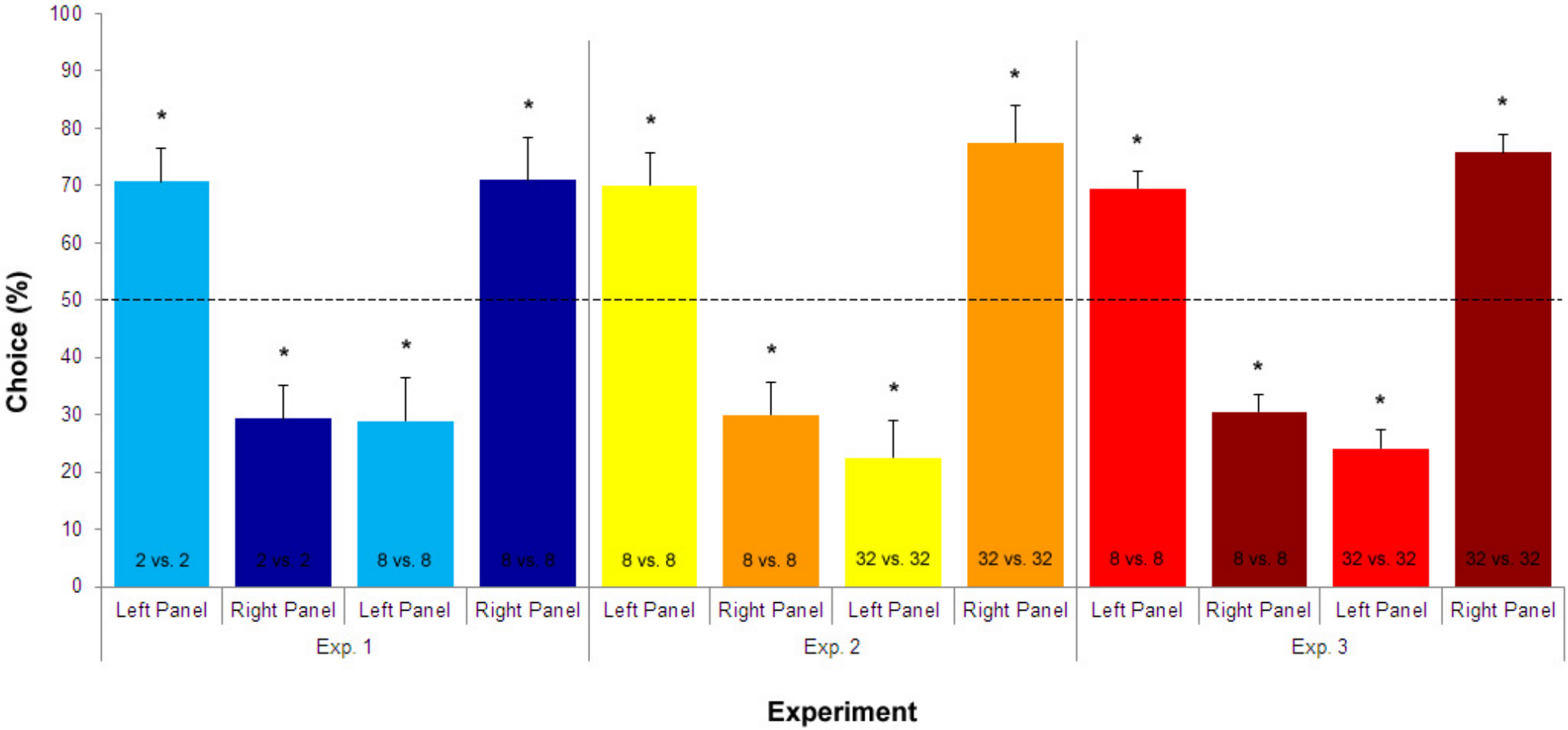


Fig. S2


100
90
80
70
60
50
40
30
20
10
0
Choice (%)
2 vs. 2
2 vs. 2
8 vs. 8
8 vs. 8
8 vs. 8
8 vs. 8
32 vs. 32
32 vs. 32
8 vs. 8
8 vs. 8
32 vs. 32
32 vs. 32
Left Panel
Right Panel
Left Panel
Right Panel
Left Panel
Right Panel
Left Panel
Right Panel
Left Panel
Right Panel
Left Panel
Right Panel
Exp. 1
Exp. 2
Exp. 3
Experiment

Experiment	Subject	First Test	Small Number Test Left trials (total valid trails)	Large Number Test Left trials (total valid trails)
Exp. 1	1	Small Number Test	4 (5)	0 (5)
	2	Small Number Test	4 (5)	3 (4)
	3	Small Number Test	1 (5)	1 (5)
	4	Small Number Test	5 (5)	0 (5)
	5	Small Number Test	4 (5)	1 (5)
	6	Small Number Test	3 (5)	0 (5)
	7	Small Number Test	2 (5)	1 (5)
	8	Small Number Test	4 (5)	4 (5)
	9	Large Number Test	4 (5)	2 (5)
	10	Large Number Test	4 (5)	3 (5)
	11	Large Number Test	2 (5)	1 (5)
	12	Large Number Test	3 (5)	0 (5)
	13	Large Number Test	4 (5)	2 (5)
	14	Large Number Test	4 (5)	0 (5)
	15	Large Number Test	5 (5)	3 (5)
Exp. 2	1	Small Number Test	4 (5)	0 (5)
	2	Small Number Test	4 (5)	1 (5)
	3	Small Number Test	3 (5)	0 (5)
	4	Small Number Test	3 (5)	4 (5)
	5	Small Number Test	2 (5)	1 (5)
	6	Small Number Test	5 (5)	1 (5)
	7	Large Number Test	3 (5)	1 (5)
	8	Large Number Test	4 (5)	1 (5)
	9	Large Number Test	5 (5)	1 (5)
	10	Large Number Test	3 (5)	0 (5)
	11	Large Number Test	4 (5)	1 (5)
	12	Large Number Test	2 (5)	2 (4)
Exp. 3 Condition 1	1	Small Number Test	4 (5)	0 (5)
	2	Small Number Test	4 (5)	0 (5)
	3	Small Number Test	4 (5)	0 (5)
	4	Small Number Test	2 (5)	0 (5)
	5	Small Number Test	3 (5)	0 (5)
	6	Small Number Test	4 (5)	2 (5)
	7	Small Number Test	5 (5)	2 (5)
	8	Large Number Test	3 (5)	1 (4)
	9	Large Number Test	3 (5)	1 (5)
	10	Large Number Test	2 (5)	2 (5)
	11	Large Number Test	3 (5)	2 (5)
	12	Large Number Test	3 (5)	1 (5)
Exp. 3 Condition 2	1	Small Number Test	3 (5)	2 (5)
	2	Small Number Test	4 (5)	3 (5)
	3	Small Number Test	3 (5)	0 (5)
	4	Small Number Test	4 (5)	1 (5)
	5	Small Number Test	5 (5)	1 (5)
	6	Small Number Test	2 (5)	0 (5)
	7	Large Number Test	4 (5)	2 (5)
	8	Large Number Test	4 (5)	2 (5)
	9	Large Number Test	3 (5)	3 (5)
	10	Large Number Test	5 (5)	1 (5)
	11	Large Number Test	3 (5)	0 (5)
	12	Large Number Test	3 (4)	2 (5)

Experiment	Subject	First Test	Small Number Test Left trials (total valid trails)	Large Number Test Left trials (total valid trails)
Exp. 3 Condition 3	1	Small Number Test	2 (5)	0 (5)
	2	Small Number Test	4 (5)	1 (4)
	3	Small Number Test	4 (5)	0 (5)
	4	Small Number Test	2 (5)	3 (5)
	5	Small Number Test	3 (5)	1 (5)
	6	Small Number Test	4 (5)	3 (5)
	7	Small Number Test	5 (5)	1 (5)
	8	Large Number Test	4 (5)	2 (5)
	9	Large Number Test	4 (5)	2 (4)
	10	Large Number Test	3 (5)	2 (5)
	11	Large Number Test	4 (5)	0 (5)
	12	Large Number Test	3 (4)	1 (5)
	13	Large Number Test	2 (5)	2 (5)

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