

Title: Object discrimination through active electrolocation: Shape recognition and the influence of electrical noise

Authors: Sarah Schumacher¹, Theresa Burt de Perera², and Gerhard von der Emde¹

Affiliations: ¹Institut für Zoologie, Universität Bonn, Endenicher Allee 11-13, 53115 Bonn, Germany;

²Department of Zoology, University of Oxford, South Parks Road, OX1 3PS, Oxford, United Kingdom

Corresponding author: Sarah Schumacher, Institut für Zoologie, Universität Bonn, Endenicher Allee 11-13, 53115 Bonn, Germany, +49 228/736159, sarah51@uni-bonn.de

Abstract

The weakly electric fish *Gnathonemus petersii* can recognise objects using active electrolocation. Here, we tested two aspects of object recognition; first whether shape recognition is dependent upon movement of the fish, and second whether object discrimination is affected by the presence of electrical noise from conspecifics. (i) Unlike other object features, such as size or volume, no parameter within a single electrical image has been found that encodes object shape. We investigated whether shape recognition is facilitated by movement-induced modulations (MIM) of the set of electrical images that are created as a fish swims past an object. Fish were trained to discriminate between pairs of objects that either created similar or dissimilar levels of MIM of the electrical images. As predicted, the fish were able to discriminate between objects up to a longer distance if there was a large difference in MIM between the objects than if there was a small difference. This suggests an involvement of MIMs in shape recognition. (ii) Electrical noise might impair object recognition if the noise signals overlap with the EODs of an electrolocating fish. To avoid jamming, we predicted that fish might employ pulsing strategies to prevent overlaps. To investigate the influence of electrical noise on discrimination performance, two fish were tested either in the presence of a conspecific or of playback signals and the electric signals were recorded during the experiments. The fish were surprisingly immune to jamming by conspecifics: While the discrimination performance of one fish dropped to chance level when more than 22 % of its EODs overlapped with the noise signals, the performance of the other fish was not impaired even when all its EODs overlapped. Neither of the fish changed their pulsing behaviour, suggesting that they did not use any kind of jamming avoidance strategy.

Keywords: Object recognition, *Gnathonemus petersii*, shape recognition, active electrosensing, jamming avoidance

1. Introduction

Weakly electric fish, *Gnathonemus petersii*, possess multiple senses, which can provide information about objects within the environment. Besides vision and the lateral line systems these fish can use active electrolocation to obtain object information. During active electrolocation the fish use object-evoked changes in a self-generated electric field (Lissmann and Machin, 1958) to detect and recognise objects (von der Emde et al., 2010). To achieve this, *G. petersii* emit brief weak electric pulses called electric organ discharges (EODs) at a highly variable rate (Carlson, 2002; Moller, 1980; von der Emde, 1992). Each EOD builds up an electric field around the fish, which is perceived locally by mormyromast electroreceptor organs. Nearby objects with different electrical properties than the surrounding water distort the electrical field leading to changes of the locally perceived EOD, forming an “electrical image” of the object on the fish’s skin (Caputi et al., 1998; Rasnow, 1996).

Although the detection and recognition of objects through active electrolocation in *G. petersii* has been studied extensively, many open questions remain concerning the parameters that enable the recognition of objects. In this study we investigated two aspects of object recognition in *G. petersii*: (1.1.) whether the recognition of object shape is dependent upon the modulations of a series of self-induced electric images that are created as a fish swims past an object, and (1.2.) whether object recognition is influenced by electrical noise.

1.1. Recognition of object shape:

While object properties such as object resistance, capacitance, size, volume, distance and location can be linked to certain combinations of parameters within the electric image (von der Emde, 2006), so far no combination has been found that would allow object shape to be encoded. One possibility is that the fish might recognise shape by engaging in movements relative to the object. Inspecting an object from different angles will modulate the successive electrical images (Hofmann et al., 2013a; Hofmann et al., 2013b) and the magnitude of these modulations will depend on the shape of the

object. Electric imagers of objects depend on several parameters, in particular on the distance of the fish from the object and on the part of the fish (flank, head, etc.) that faces the object. Nevertheless a few general statements regarding the differences of electric images of spheres and cubes on the one hand and elongated objects (ellipsoid) can be made. For example, when perceived from a constant distance, the electric images of a sphere are constant for each angle of perspective, while the electric images of an elongated object, e.g. an ellipsoid, differ depending on whether it is perceived facing its longer or its shorter side. Therefore, the modulations in a series of electrical images that are induced by a fish swimming past an object might provide useful information that could be used to recognise object shape. We define the changes in the electrical images that occur as a fish swims past an object, as movement-induced modulations (MIM). Here we tested whether movement-induced modulation is used to encode object shape by comparing the performance of the fish when they had to discriminate between two objects (a cube and a sphere), which both evoked similar levels of MIMs, or between objects (an ellipsoid (presented with its longer side facing the observation gate) and a sphere), which produce very different MIMs. If the fish indeed use the difference in MIM for object shape discrimination, we would predict that it should be easier for them to discriminate between the sphere and the ellipsoid than to discriminate between the sphere and the cube. Accordingly, discrimination between the sphere and the ellipsoid should be possible up to a greater distance compared to discrimination between the sphere and the cube.

1.2. Influence of electrical noise on object recognition

In their natural environment, *G. petersii* are confronted with electrical noise during active electrolocation, e.g. arising from other nearby electric fish also emitting EODs. In contrast to gymnotiform pulse-type electric fish, mormyrid weakly electric fish can clearly separate their own EODs from those of other nearby fish as long as they do not overlap, by using their corollary discharge (Bell, 1989). However, when temporally overlapping with a foreign signal, the waveform and amplitude of the fish's own EOD can be changed. These noise related changes in the electric field could potentially mask or jam the object evoked changes and thus interfere with object recognition (Heiligenberg, 1974, 1976). While there are many investigations into the jamming avoidance response of South American wave-type electric fish (for example (Watanabe and Takeda, 1963), however,

relatively little is known about how the African pulse-type Mormyridae cope with electrical noise (Heiligenberg, 1974, 1976; Moller and Bauer, 1973; Westby, 1981).

A possible mechanism for avoiding jamming in mormyrids could be the so-called echo response, during which one fish emits its EODs with a preferred short (usually 10-14 ms) latency after the EODs of another fish (Heiligenberg, 1976; Russell et al., 1974; Schuster, 2001). Pulsing with a latency of ca. 10 -14 ms decreases the probability of overlaps, because the non-focal fish is unlikely to emit another EOD in this timeframe. The echo response is described in many different species of pulse-type electric fish, but in addition to being a possible jamming avoidance response, it is also described as an electrical communication behaviour (Arnegard and Carlson, 2005; Gebhardt et al., 2012; Heiligenberg, 1976; Lückner and Kramer, 1981; Russell et al., 1974). Here, we tested whether electrical noise, either from conspecifics or artificial electrical signals, influences the object discrimination performance of *G. petersii*. Furthermore, we recorded the electrical signals emitted during object discrimination to investigate whether the fish used any type of jamming avoidance response.

2. Material and Methods

2.1. Animals and set up

During our experiments we used four experimental fish of the species *Gnathonemus petersii* (two for shape recognition (fish 1 and 2) and two for the influence of electrical noise (fish 3 and 4)). Two additional fish of the same species were used to serve as "jamming fish" (fish 5 and 6). The experimental fish were kept individually in tanks (75 cm x 40 cm x 40 cm), which also served as an experimental arena. The jamming fish were kept in separate housing tanks (75 cm x 40 cm x 40 cm) and were only put into the experimental tanks during the experiments. The water conditions in all tanks were kept constant with a temperature of $26 \pm 1^\circ\text{C}$, a pH-value of 7 ± 0.5 and a conductivity of $100 \pm 10 \mu\text{S/cm}$. The artificial dark:light-cycle was set to 12:12h. All experiments except for the dark controls were conducted under ambient light level of ca. 65 lux (measured just above the water surface). Under these bright light conditions the ability of *G. petersii* to discriminate between objects visually deteriorates (Schuster and Amtsfeld, 2002).

The experimental tanks were divided into two compartments (40 cm x 40 cm, 35 cm x 40 cm) with a partition containing two gates (Fig. 1). The smaller compartment was used as the living area of the fish and contained hiding places, while the bigger compartment served as experimental area, which was again divided into two sections with a divider. During training, an object was placed 1 cm behind each gate. In order to ensure that the fish kept this minimal distance to the object, distance grids, made of a plastic frame stringed with thin cotton threads (mesh size 15 mm diagonal), were placed directly behind the gates (between gate and object). These grids allowed unimpaired electrolocation but in order to pass them, the fish had to push them aside.

The fish were trained individually in a two-alternative forced-choice procedure (2AFC) to swim through the gate with the positive object behind (associated with a food reward) and to avoid the gate with the negative object behind (associated with a mild punishment of being chased back into the living area). The position of the positive object was changed pseudo-randomly after Gellermann (Gellermann, 1933). Each fish conducted 20 – 40 trials per training day.

2.2. Training groups:

The fish were divided into two different training groups. Although all fish underwent the same principal training procedure described above, the different groups were trained with different objects and under different conditions.

2.2.1. Recognition of object shape:

Two naive fish were trained to discriminate between two aluminium objects, which only differed in shape. In the first training phase, fish 1 was trained to discriminate between a sphere (\varnothing 3 cm, S+) and a cube (side length 2.42 cm, S-, presented with its side directly facing the door) and fish 2 was trained to discriminate between the sphere and an ellipsoid (length: 4.78 cm, \varnothing : 2.39 cm, S-, presented with its longer side facing the door.) (Fig. 1). After the fish reached a pre-assigned learning criterion of 75% correct choices on three consecutive training days, test trials were introduced every third trial, during which the distance of the object to the distance grids was varied up to a distance of 4 cm. The distance was chosen pseudo-randomly for each test trial. During tests the fish were neither punished nor rewarded to avoid reinforcement.

Subsequent to this first test series, a second training phase was conducted during which the negative objects were exchanged, because previous studies have shown that during the two-alternative forced-choice experiments the fish learn to recognise and avoid the negative object rather than to swim to the positive object. If the negative object was exchanged with an unknown object the fish were unable to fulfil the task, while an exchange of the positive object did not impair the discrimination performance. From this we can conclude that the ability to fulfil the task depends on the recognition of the negative object. Fish 1 was now trained to discriminate between the sphere and the ellipsoid and fish 2 was trained with the cube as negative object. After the fish had reached the learning criterion of 75% correct choices on three consecutive training days, again, they were subjected to a second test series with varying object distances conducted in the same way as in the first test series.

Subsequently to the second test series, control tests were conducted at 1 cm distance to ensure that the fish did not use visual (dark control) or lateral line (agar control) cues to discriminate between the objects. During the agar control the objects were encased in a cube of electrically transparent agarose, which had approximately the same conductivity as the surrounding tank water. To achieve this, a mixture of 10 ml tap water, 90 ml deionised water and 2 g Agarose powder was boiled (Agarose BP 160-100, Fisher Scientific, Fair Lawn, New Jersey, USA) cast in moulds. During the dark control the experiments were conducted in complete darkness (<0.01 lux). A video camera with night shot function was used to observe the outcome of the trials. Infrared light of 850 nm, which is invisible for *G. petersii*, was used to illuminate the tank (IR Illuminator, S8030-3D-L-IR, ITAKKA, Wattens, Austria). In fish 1 both controls were conducted with the ellipsoid as negative object and in fish 2 they were conducted with the cube as negative object.

To compare the performances between the tests containing the two different negative objects, the percentage of correct choices was calculated for all object distances for each fish. The performance for each distance was tested against chance-level with a χ^2 -test for both negative objects and the exact Fisher-test was used to test the results with the different negative objects against each other for each distance. The distance discrimination threshold was determined by calculating the intersection of the applied sigmoidal fit with the pre-assigned 70% threshold level. Furthermore the performance during training was compared with the performances during the control tests using the exact Fisher-test.

2.2.2. Influence of electrical noise on object recognition:

The standard set up described above was slightly modified by adding a cage for confining the jamming fish (4.5 cm wide, 17 cm long, 40 cm high) to the partition dividing the experimental area between the both gates. To record the electrical signals, two pairs of silver electrodes were attached crosswise to the inner side of the tank walls. The recorded signals were amplified by a differential amplifier (electronic work shop University of Bonn) and digitalized with an analog-to-digital converter (Micro 1401, CED, Cambridge Electronic Designs). The digitalized data was then saved and analyzed on a computer with the program Spike2 (Spike 2 Version 5.00, CED). The experiments were also recorded with a video camera (HDR-HC3EK, Sony) that was placed 85 cm above the tank.

During training without electrical noise two fish were trained to discriminate between two aluminium objects, which either differed in size (small cube ($2 \times 2 \times 2 \text{ cm}^3$) vs. large cube ($3 \times 3 \times 3 \text{ cm}^3$), fish 3) or in shape and volume (pyramid (base: $3 \times 3 \text{ cm}^2$, height: 3 cm) vs. large cube, fish 4). The fish had been used previously in similar experiments with the same objects. After the fish had learned the task and reached a learning criterion of 70% correct choices on three consecutive training days, experiments with electrical noise (natural and artificial) were introduced. Each day 40 trials were conducted with each fish. 10 trials were conducted with and 30 trials were conducted without the noise signals. Since the noise trials could not be interspersed pseudo-randomly, the trials without noise were either conducted 100% before, 100% after, or 50% before and 50% after the trials with noise. In addition to the trials at 1 cm distance, test trials without reward or punishment were conducted at 3 cm distance.

To introduce natural electrical noise a conspecific (jamming fish) was placed in the cage between the gates during the experiments. Before the experiments started the fish were given 5 min to acclimate. During this time the experimental fish could move freely within the whole tank without objects or distance grids present.

To test the influence of artificial electrical noise, playback experiments were conducted. During these trials a recorded and digitalised EOD of *G. petersii* was played back to the fish with varying frequencies (100 Hz, 200 Hz, 250 Hz (fish 3), 100 Hz, 500 Hz and 1.5 kHz (fish 4)). In addition, white noise was used as a jamming signal in both fish. To produce these signals, two carbon electrodes were placed inside the cage between the gates with a distance of 7 cm to each other. The signals

were generated with a waveform generator (G5100A 50 MHz Function/Arbitrary Waveform Generator; Picotest CXI), which stored the digitalised EOD.

An agar control and a dark control (for set up description see 2.2.1) were conducted with fish 4 to ensure that neither the lateral line system nor vision were used to discriminate between the objects. Both controls were conducted under the presence of playback noise signals with a frequency of 1.5 kHz at 1 cm and 3 cm distance.

To compare the performance of each fish with and without the electrical noise, the percentage of correct choices was calculated for each condition at 1 cm and 3 cm object distance and plotted in a bar chart. The Chi²-test was conducted to test whether the performance was significantly different from chance level and the exact Fisher-test was used to test whether the results with the different noise signals were significantly different from the result without noise and whether there was a significant difference between the results during training and the control tests.

2.2.2.1. Electrical signals

To analyse whether *G. petersii* uses any strategies to avoid jamming by electrical noise, the electrical signals within the experimental tank were recorded during all experiments. A period from three seconds before opening the gates to three seconds after passing the gates was analysed. This timeframe was divided into three phases, which were analysed separately: Before the trial (the three seconds before opening the gates), during object inspection (from opening the gates till swimming through the gates) and after the trial (the three seconds after swimming through the gates). Twelve trials with natural electrical noise and four trials for each artificial noise signal were pseudo-randomly chosen and analysed for each fish.

For the experiments with natural electrical noise, each recorded EOD had to be assigned to the fish that emitted it. Since the polarity and the amplitude of the recorded EOD depends on the position of the fish relative to the recording electrodes, this was achieved by comparing the positions of both fish relative to the recording electrodes on the video recording at the time of each recorded EOD. The occurrence time of each EOD was exported to a Microsoft Excel file. The inter discharge interval (IDI) of the EODs of each fish, or of the playback signals was calculated and plotted over time for each trial. Each trial with natural noise signals was also tested for synchronisation of the two fish.

Synchrony was defined as a longer period of time involving several consecutive EODs, during which two fish discharge their EODs in a time-locked or phase-locked manner with a short response duration. For the analysis a cross-correlation diagram was generated as described in Gebhardt et al., (2012) using Matlab R2009a (The MathWorks, Inc.; www.mathworks.com).

In the second analysis, the data of each experimental phase (before the trial, during inspection of the objects and after the trial) of all trials was pooled for each noise signal. The response intervals of the experimental fish relative to the noise signals (natural and artificial) were calculated (time between a noise pulse and the pulse of the experimental fish), plotted in histograms (bin width 2 ms) and analysed for echo responses. An echo response was defined as an increased relative frequency of EOD responses with a short time delay less than 30 ms. The recorded distribution of response intervals was compared to the randomly expected distribution that would have occurred by chance, which was calculated based on the individual IDI pattern of the leading noise signal. Accordingly, the response intervals of the noise fish to the experimental fish were analysed.

Within the pooled data the number of EODs, which overlapped with the noise signals, was counted and the relative frequency of overlaps was calculated. For the experiments with natural noise signals (conspecific), the results were plotted in a bar chart for each phase of the experiment. As a reference, the randomly expected number of overlaps was determined using the statistics software R 2.7.1 (The R Foundation for Statistical Computing). The applied program created 50 random EOD-patterns with the same frequency, EOD-duration and minimal observed IDI as the fish and calculated the mean number of overlaps and its standard deviation.

For the experiments with artificial noise signals, the randomly expected relative frequency of overlaps was determined by calculating the frequency of the noise signal, at which 100 % of the EODs of the experimental fish had to overlap with the noise signal and connecting this point with 0. The resulting line gave the linear relation between the frequency of the noise signal and relative frequency of overlaps. The noise frequency, at which 100 % of the EODs overlapped, was calculated by adding up the duration of the fish's EOD (0.5 ms) with the duration of the playback EOD (0.7 ms) and taking the inverse of this value to receive the frequency ($1/0.012 \text{ s} = 833.33 \text{ Hz}$).

In order to receive a reference without interaction between the fish and the noise signal, separately recorded signals of the experimental fish and the jamming fish or the playback signal were overlaid in the computer. To do this, the experimental fish was recorded during a trial, during which no

electrical noise signals were presented, and the jamming fish was recorded during a sham trial (same experimental set up and same procedure but without an experimental fish) and both records were overlaid so that the point of opening the doors matched. These non-interacting signals were analysed as described above.

3. Results

3.1. Recognition of object shape:

Fish 1 was first trained to discriminate between a sphere (S+) and a cube (S-) and then its discrimination performance was tested at varying object distances. Subsequently, the same fish was trained using an ellipsoid as the S- and tested again with the objects at varying distances to the grids. Both fish showed a constant behaviour during object inspection in which they inspected both objects from a very short distance from the distance grids by moving from one end of the gate to the other whilst facing the object. The fish either repeated this behaviour several times or made their decision after the first inspection. Fish 1 was able to discriminate between the sphere and the cube up to a distance of 2 cm, reaching a performance significantly different from chance level (Fig. 3 A). At a distance of 3 cm, the performance dropped to 50 % chance level. With the ellipsoid as the negative object, the performance at the same distance was significantly higher than with the cube, and the performance dropped to chance-level at a distance of 4 cm.

Fish 2 was first trained to discriminate between the sphere (S+) and the ellipsoid (S-) and after the distance tests were conducted, it was trained with the cube as the S-. With both negative objects this fish reached a performance significantly different from chance-level up to 2 cm (Fig. 3 B). However at this distance of 2 cm, the performance with the cube as S- was below 70 % and significantly lower than with the ellipsoid.

To further compare the performances with the cube and with the ellipsoid as negative objects, the distance thresholds for both objects were calculated. In both fish, the distance threshold for the experiments with the cube as negative object was about 1 cm shorter than with the ellipsoid (Fig. 4). With 2.29 cm and 3.30 cm for fish 1 and 1.76 cm and 2.75 cm for fish 2, the distance thresholds for the discrimination of objects that only differ in shape are relatively small compared to the distance

thresholds of experiments with objects that differed in size (3.9 cm) and objects that differed in shape and volume (3.9 cm) (von der Emde et al., 2010).

To ensure that the fish was using the active electric sense to discriminate between the objects, both the influence of the lateral line system and vision were excluded (lateral line: objects encased in cubes of electric transparent agarose, vision: experiments conducted in complete darkness) during control experiments. The results of these control tests show that the performance of both fish did not change significantly compared to the performance under training conditions.

3.2. Influence of electrical noise on object recognition

3.2.1. Discrimination performance:

Fish 3 was trained to discriminate between objects that differed in size (small and large cube) and fish 4 was trained to discriminate between objects that differed in shape and volume (pyramid and large cube). After the fish had learned the task they were tested with and without electrical noise signals (natural and artificial) at 1 cm and 3 cm object distance. Without noise signals, both fish reached a performance of over 90 % correct choices when the objects were placed 1 cm behind the gates and, although the performance decreased, both fish were still able to discriminate between the objects with an object distance of 3 cm (Fig. 5).

When electrical noise was presented to the fish during the experiments the performance of both fish differed. The performance of fish 4 was not affected by any of the noise signals presented, neither when the objects were placed 1cm behind the gates nor when they were presented at a distance of 3cm. In fish 3, however, the performance at a distance of 1 cm decreased significantly and dropped to chance-level when artificial noise in form of playback signals with a frequency of 250 Hz was presented. With the increased object distance of 3 cm, the playback signal with a frequency of 200 Hz and the white noise, which both did not influence the performance at 1 cm object distance, led to a significant drop of performance, which signals an inability to discriminate between the objects under these conditions. The discrimination performance of fish 3 significantly decreased when a conspecific was present during the experiments to a level of about 79 %. However further analyses of these experiments showed that this was only a temporal effect. During the first 10 experimental days

the performance dropped to a level of ca. 60 % correct choices but subsequently increased again to the same level as during experiments without any electrical noise.

To ensure that the fish used the active electric sense to discriminate between the objects even under jamming conditions, control experiments with the 1.5 kHz playback signal were conducted with fish 4 at 1 cm and 3 cm. During these tests, the fish could not use the lateral line system (objects encased in electrically transparent agarose) or vision (tests conducted in complete darkness) to discriminate between the objects. The results show that the performance during both control tests did not significantly differ from the performance during tests under standard conditions, showing that the fish was still able to discriminate between the objects under jammed conditions, when vision and the lateral line system could not be used for the task (Fig.7).

3.2.2. Electrical signals:

To determine whether the fish applied any strategies to avoid interference with the electrical noise signals, the EODs of the fish were recorded during the trials and analysed. The inter-discharge-intervals (IDI) of the experimental fish and of the jamming fish were calculated and plotted over time to determine whether there were typical reoccurring discharge patterns during the experiments. During most of the analysed trials (40 trials for fish 3 and 36 trials for fish 4), both experimental fish showed the same typical discharging pattern, which is shown as an example in one trial of fish 3 in Fig. 8 A1. After the doors were opened (indicated by the red line), the fish decreased the IDIs for a period of about 250 ms to ca. 10 ms (minimal measured IDI 7.5 ms). Except for this brief decrease of IDIs, the fish discharged with more or less constant IDIs around 20 ms. This pattern also occurred during trials where no noise signal was present (Fig 8 B1, both fish were recorded separately and the recorded tracks were overlaid on the computer), showing that this behaviour was not induced by the electrical noise. The discharging behaviour of the both jamming fish was much more variable with longer IDIs.

The cross-correlation analysis of all analysed trials (18 trials for each fish) showed only very slight or no synchrony of both fish (example of fish 3 in Fig. 8 B1). The comparison to the separately recorded trials without interactions between the fish showed that these very slight synchronisations also occurred in these trials. Therefore these minimal synchronisations were probably not due to active interactions between the fish and so were irrelevant to our study.

The cross-correlation diagrams show interactions between the fish and the noise signals, only if several consecutive EODs are time- or phase-locked. Therefore to determine whether the fish might have preferred certain response intervals independent of the time of occurrence, the response interval histograms were analysed for echo responses. Neither experimental fish ever showed an increased frequency of any response interval in any of the experimental phases (before the trial, during inspection, after the trial) no matter which form of noise signal was presented. The observed distribution of response intervals matched the randomly expected results in all experiments with only very small deviations (Fig. 9 A, C, E; Fig. S1 A, C, E). The comparison to the results of trials during which the experimental fish was recorded without any noise signals but the noise signals were added in the computer (Fig. 9 B, D, F; Fig. S1 B, D, F), shows that deviations from the randomly expected distribution also occurred within the same range without interaction between the experimental fish and the noise signal. This suggests that the fish did not actively alternate their response intervals.

However analyses of the response intervals of both jamming fish show that after the trial the response intervals of 10 to 12 ms (jamming fish of fish 3) respectively 10 to 14 ms (jamming fish of fish 4) occurred with an increased frequency (Fig. 10 E, Fig. S2 E). The jamming fish emitted nearly 20 % (jamming fish of fish 3) respectively 30 % (jamming fish of fish 4) of its EODs with these response intervals to the EODs of the experimental fish. No such large deviations to the expected results were found when the fish were recorded separately. Furthermore, there was a slight increase of the frequency of response intervals of 10 -12 ms respectively 10 to 14 ms during the experimental phase, during which the experimental fish inspected the objects (Fig 10 C, Fig S2 C). Before the trial started no increased frequency of any response interval was observed.

The relative frequency of overlapping EODs of the experimental fish with the EODs of the jamming fish showed that only 1.23 % to 2.25 % of all EODs of the experimental fish were subject to overlaps (Fig. 11 A, C). Since the number of overlaps depends of the pulse frequency of both fish, which was very variable, the different phases and the different conditions cannot be compared directly. Therefore the randomly expected frequency of overlaps was used as a reference. In both experimental fish, the relative frequency of overlaps laid within the standard deviation of the randomly expected results, suggesting that there was no active reduction of overlaps. The comparison with the results of the separately recorded experiments (Fig. 11 B, D) shows that these values deviate within a similar or even slightly larger range from the randomly expected values, suggesting that these kind of deviations are due to slight inaccuracies within the modulation.

Analysis of the frequency of overlapping EODs of the experimental fish and the artificial noise signals showed that in both fish the relative number of overlaps was very close to the expected values during all three phases of the experiment (Fig.12). Furthermore, the comparison of the number of overlaps during trials with playback signals (Fig. 12 A, C, E), with the results of the trials, during which the fish was recorded without noise and the noise signals were added afterwards on the computer (Fig. 12 B, D, F), showed that there was no difference in the deviation from the expected results between both conditions.

4. Discussion

In this study we investigated the influence of object shape and electrical noise on the object discrimination performance of *Gnathonemus petersii*. During active electrolocation most object features are linked to a certain parameter or combinations of parameters within the electrical image and can be extracted from the object evoked changes of a single EOD (von der Emde, 2006). This enables the fish to recognise these features very rapidly and provides the active electric sense with a very high temporal resolution of up to over 100 Hz. However, the recognition of object shape seems to be more complex, because so far no parameter within the electrical image has been found that encodes shape information directly or through combinations of parameters. Therefore shape recognition might not rely on the information extracted from a single electric image but might depend on (or at least might be improved through) the analysis of the movement induced temporal modulation of a series of electric images (Hofmann et al., 2013a; Hofmann et al., 2013b). To test this, we used a discrimination paradigm in which two different negative objects (an ellipsoid, presented with its longest side towards the fish, and a cube) produced different magnitudes of movement induced modulations (MIM). At close range the MIMs evoked by both negative objects clearly differ from those evoked by the sphere. However due to the elongated shape of the ellipsoid, the difference between the MIMs of the electric images created by the ellipsoid and those of the sphere are greater than the differences between the MIMs of the cube and the sphere. Since the differences in the MIMs of the different objects decrease with increasing object distance, the fish should be able to discriminate between the sphere and the ellipsoid up to a greater distance than between the sphere and the cube. The results of our experiments show that both fish were able to discriminate between the sphere and the ellipsoid up to greater distance than between the sphere and the cube (Fig. 3). This corresponds with the

predicted results, supporting the hypothesis that movement-induced modulations of the electric image of an object play an important role during shape recognition. The stereotypical behaviour of the fish during inspection, moving in front of the object, suggests that the fish use this behaviour to obtain the information necessary for the discrimination task.

Furthermore, the results of the distance discrimination thresholds (Fig. 4) show that object discrimination based on shape information is restricted to shorter distances when compared to previous studies in which the fish could use other object features such as volume and size (von der Emde et al., 2010). This could be due to individual performance differences between the fish, however results of another study investigating object discrimination with different objects that only differed in shape show that none of the fish were able to discriminate between the objects at a distance greater than 2.5 cm using the electric sense (Schumacher et al., 2016). Together, these results support the hypothesis that extracting information about shape from the electrical image is more difficult than extracting information about volume or size.

In the second experimental series, we investigated the influence of electrical noise on object discrimination performance of *G. petersii*. The results of our experiments with natural and artificial electrical noise stimuli show that while the performance of one fish was impaired through artificial playback signals with a frequency of more than 200 Hz, the second fish showed no change in performance even when playback noise signals were presented with a frequency of 1.5 kHz (Fig 6). The different influence of the noise signals on the performance of the fish might be explained by differences in size of the fish and the resulting differences in the amplitude of the EODs. Fish 4, which showed no change in performance, was ca. 1.5 cm bigger than fish 3 and also the EOD amplitude of fish 4 was slightly larger.

Another explanation for the decrease in performance of fish 3 could be that it was due to distraction rather than to real interference by the electrical noise. During the first few weeks of the experiments with the conspecific as the natural noise source, fish 3 showed changes in its behaviour during the trials. The fish often did not collect its reward and instead tried to attack the jamming fish within the cage. Further analyses of the discrimination performances showed that during this time the discrimination performance during trials with the conspecific was significantly decreased. After approximately three weeks of experiments, the fish no longer showed the aggressive behaviour and the discrimination performance in trials with natural noise increased to the same level as during trials

without noise. Also during trials with the artificial playback noise, fish 3 showed signs of aggression towards the noise source although these attacks were not as strong as those to the jamming fish. This behaviour suggests that fish 3 might have not paid full attention to the objects and the experiments but rather was distracted by the presence of another fish within its territory. In comparison, fish 4 showed far less aggressive behaviour, and indeed this only occurred after the trial was completed. During the trial, this fish showed no changes in the behaviour compared to trials without noise.

Surprisingly, our results show that the presence of electrical noise affects active electrolocation performance very little. Fish 4 was never impaired in its performance and fish 3 was affected only with playback signals of a frequency more than 200 Hz. Such a high frequency of jamming stimuli probably occurs in nature very rarely, if at all. The highest discharge rate of a single individual of *G. petersii* is always well below 200 Hz (Bauer and Kramer, 1974; Kramer, 1979) and to reach a jamming frequency of 200 Hz, several individuals would have to be in close proximity to an electrolocating fish simultaneously. Therefore, it can be assumed that *G. petersii* will not experience any jamming of its own electric signals in nature. Even in the presence of other fish they still can engage in active electrolocation.

The analysis of the electrical signals recorded during the trials showed that both fish used certain EOD patterns during the experiments (Fig. 8). When the doors were opened both fish increased their pulse frequency for a short period of time (250 ms) up to 133 Hz. With this novelty response the fish increased the temporal resolution of the electric sense and was thus able to gather more information about the objects within a short timeframe. Since producing EODs with such a high frequency is energetically costly, the duration of such novelty responses is restricted to only short periods of time. This typical discharging pattern however was independent of the presence of electrical noise and occurred in both fish during trials with and without noise. The novelty response could either be a reaction of the fish to the sudden opening of the gates or a specific reaction of the fish to the experiments. The so-called novelty response in *G. petersii*, a transient acceleration of the EOD rate, is usually described without a discrimination context but as a reaction to any novel sensory stimulus (Post and von der Emde, 1999), suggesting that during our experiments it might have been triggered by the sudden opening of the doors. However, tests, during which no objects were placed behind the gates and without the usual preparations before a trial, the fish did not show novelty responses after opening the doors. Also the jamming fish did not react with novelty responses to opening the doors.

This suggests that this behaviour might have some experimental context, enabling the fish to gather information about the objects rapidly.

Neither of the two experimental fish showed any changes in their pulsing behaviour during trials with electrical noise nor did they show any synchronisation of the discharging behaviour with the jamming fish (Fig. 8, 9, 11, 12 and S1). The analysis of the response interval histograms of both experimental fish (Fig. 9 and S1), show no increased relative frequency of any response interval, suggesting that the fish did not use echo responses as a jamming avoidance response. This result is supported by the relative frequency of overlapping EODs, which showed no relevant deviations to the randomly expected results during trials with any form of noise signal (Fig. 11 and 12). This suggests that the fish did not apply a specific strategy to avoid or reduce EOD overlaps. This result does not correspond with results in *Brienomyrus* a related genus of pulse-type electric fish (Heiligenberg, 1976). Experiments with these fish suggest that they use echo responses to avoid EOD overlaps. The lack of any jamming avoidance strategy in *Gnathonemus* may be due to the fact that, in comparison to *Brienomyrus*, adults of this species are less social and usually do not live in groups as adults (Moller, 1995). Thus *G. petersii* may not encounter many natural situations where they are jammed by electrical noise from conspecifics.

The increased relative frequency of response intervals of 10 - 14 ms of the jamming fish after the trial (Fig. 10 and S2) suggests that the echo response is used as a communication device in *G. petersii*. After the trial, both fish were positioned together in the experimental area of the tank and as described above both experimental fish (especially fish 3) showed aggressive behaviour towards the jamming fish. Since the jamming fish were much smaller (ca. 4 cm) than the experimental fish and showed little or no aggression, the echo response might have been used as a submissive signal in this context. Studies with other mormyrid fish also suggest that the echo response might be used as a non-aggressive communication signal (Gebhardt et al., 2012).

During trials with natural noise signals, only 1.23 % to 2.25 % of all EODs of the experimental fish overlapped with EODs of the jamming fish (Fig. 11). Due to the high number of uncontaminated EODs, negative effects of the overlaps on the discrimination behaviour are unlikely, supporting the hypothesis that the slight decrease of discrimination performance in fish 3 during trials with the jamming fish are probably due to distraction caused by the presence of another fish. However since the results of the two experimental fish are not consistent, our experiments do not provide conclusive

information on how many overlaps would impair object discrimination. While fish 4 was able to fulfil the discrimination task successfully even when all of its own EODs overlapped with the noise signal, the discrimination performance of fish 3 was impaired when more than 22 % of its EODs overlapped with the noise signal. However, 22% overlaps is a rather high number that probably never occurs under natural conditions.

In conclusion, the results of our study suggest firstly, that during object recognition, movement induced modulations might facilitate recognition of object shape. Secondly, we show that object recognition is not impaired by electric signals of conspecifics and even the effects of noise signals, which lead to an overlap of every EOD, can potentially be compensated for without the necessity of a jamming avoidance response. These results add an important component to our understanding of the mechanisms of natural object recognition through active electrolocation in the weakly electric fish *G. petersii*.

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The experiments were carried out in accordance with the guidelines of German Law, with the animal welfare regulations of the University of Bonn, and with the “Guidelines for the treatment of animals in behavioural research and teaching”, Association for the Study of Animal Behaviour (ASAB), 2006.

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Figure captions:

Figure 1: Experimental setup used during the experiments for investigating recognition of object shape. A) Schematic top view of the setup. B)-D) Experimental objects used during the shape recognition experiments. All three objects were made of aluminium and had the same volume, thus they only differed in shape. The sphere (B) was used as the positive object during all shape recognition experiments and either the ellipsoid (C) or the cube (D) was used as the negative object.

Figure 2: Experimental setup for the experiments investigating the influence of electrical noise on the discrimination performance. A) Schematic top view of the experimental setup. The standard setup was slightly modified by adding a cage to the divider and adding two pairs of recording electrodes, which were attached crosswise to the tank walls. During the experiments with a conspecific as noise source a jamming fish was put into the cage between the objects. During the experiments with playback signals two carbon electrodes were placed 7 cm apart from each other in the cage. B) – D) experimental objects used during the electrical noise experiments. Fish 3 was trained to discriminate between the positively associated small cube (C) and the negatively associated large cube (B). The large cube also served as the negative object during the experiments with fish 4 while the pyramid (D) was used as the positive object.

Figure 3: Discrimination performance of fish 1 (A) and fish 2 (B) at different distances when trained to discriminate between a sphere and a cube (blue) and a sphere and an ellipsoid (red). Fish 1 was first trained with the cube and then with the ellipsoid and fish 2 was first trained with the ellipsoid and then with the cube. Training was conducted with 1 cm object distance and tests at longer distances were introduced once the fish had reached a learning criterion of 70% correct choices on three consecutive training days. The dashed line indicates the 50 % chance-level and the number of trial conducted with each distances is given within the bars. A χ^2 -test was conducted to test whether the performance was significantly different from chance-level (indicated by the stars within the bars; *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$). Furthermore the performance with the cube and with the ellipsoid was compared for each distance using exact Fisher-tests (*: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$).

Figure 4: Distance thresholds of fish 1 (A) and fish 2 (B) for the discrimination of a sphere and a cube (blue) and a sphere and an ellipsoid (red), which only differed in shape. Thresholds were determined by fitting the results using a sigmoidal curve and calculating the distance at which this curve intersected with the pre-assigned 70% discrimination threshold level (indicated by the dotted line).

Figure 5: Discrimination performance of fish 1 (A) and fish 2 (B) during control tests. The control tests were conducted with the cube as negative object in fish 1 and with the ellipsoid as negative object in fish 2. The performance during training (blue, red; same data as in Fig. 3) was compared with the performance during an agar control (light blue, light red) and during a dark control (dark blue, dark red). The agar control was conducted in order to exclude effects of the lateral line system by encasing both objects in a cube of electrical transparent agarose, so that both objects had the same outer shape. To exclude vision the dark control tests were conducted in complete darkness (<0.001 lux). The performance during training was compared with the performance during the agar control and the dark control using exact Fisher-tests. (n.s.: not significant) For further description see Fig. 3.

Figure 6: Discrimination performance of fish 3 (A) and fish 4 (B) during tests at 1cm and 3cm object distance without any electrical noise (blue), with natural noise emitted by a conspecific (red), with white noise (dark blue) and with playback signals with varying frequencies (shades of grey). Fish 3 was trained to discriminate between a small and a big cube and fish 4 was trained to discriminate between a pyramid and a big cube. Exact Fisher-tests were conducted to test whether there was a significant difference between the performance without electrical noise and the performances with the different noise signals. For further description see Fig. 3.

Figure 7: Discrimination performance of fish 4 during control tests at an object distance of 1 cm (left) and 3 cm (right). An agar control (light green) was conducted to exclude influence of the lateral line system and a dark control (dark green) was conducted to exclude influence of vision on the discrimination performance of the fish. Both controls were conducted in presence of electrical noise in form of a playback signal with a frequency of 1.5 kHz. The performance of the fish during tests with the 1.5 kHz playback signal under standard conditions (black, same data as in Fig. 6) is shown as a

reference. Exact Fisher tests were used to compare the performance during training with the performances during the agar control and the dark control. For further description see Fig.3.

Figure 8: Discharging behaviour of fish 3 and the jamming fish during exemplary object discrimination trials when both fish were present together in the tank (A) and when both fish were recorded separately (B). For the separate recordings in B, fish 3 was recorded during a trial, during which no electrical noise signals were presented and the jamming fish was recorded during a sham trial (same experimental set up and same procedure but without an experimental fish). The separately recorded tracks were overlaid in the computer so that the time of opening the doors matched. (A1, B1) Inter-discharge-intervals of fish 3 (black) and the jamming fish plotted over time. (A2, B2) Cross-correlation diagrams of the electric signals over time. Note that the negative correlation in A2 at about 7 s is an artefact resulting from the opposing responses of the two fish to the opening of the doors, which did also occur in separately recorded trials. The red line indicates the point of time when the doors were opened (start of trial) and the blue line indicates the point of time when the fish swam through one of the doors (decision).

Figure 9: Response interval histograms of fish 3 with a conspecific before the trial started (A, B), during inspection of the objects (C, D) and after the trial (E, F). The fish were either recorded when together in the experimental tank (A, C, E) or were recorded separately and the records were overlaid on the computer (B, D, F). The red line indicates the randomly expected distribution based on the individual pulse pattern of the jamming fish.

Figure 10: Response interval histograms of the jamming fish 5 in response to experimental fish 3 before the trial started (A, B), during inspection of the objects (C, D) and after the trial (E, F). For further description see Fig. 9.

Figure 11: Relative frequency of overlaps of the EODs of the experimental fish with EODs of the jamming fish of fish 3 (A, B) and fish 4 (C, D) during the different experimental phases (before the trial

started (dark grey), during inspection of the objects (grey) and after the trial (light grey)). The fish were either recorded together (A, C) or were recorded separately and the records were overlaid on the computer (B, D). The mean relative frequency of overlaps of 50 randomly shuffled simulations is given as a reference (red bars). The error bars indicate the standard deviation. Since the number of overlaps depends on the pulse frequency of the jamming fish, the results are only directly comparable with the respective randomly expected values and not between the bars.

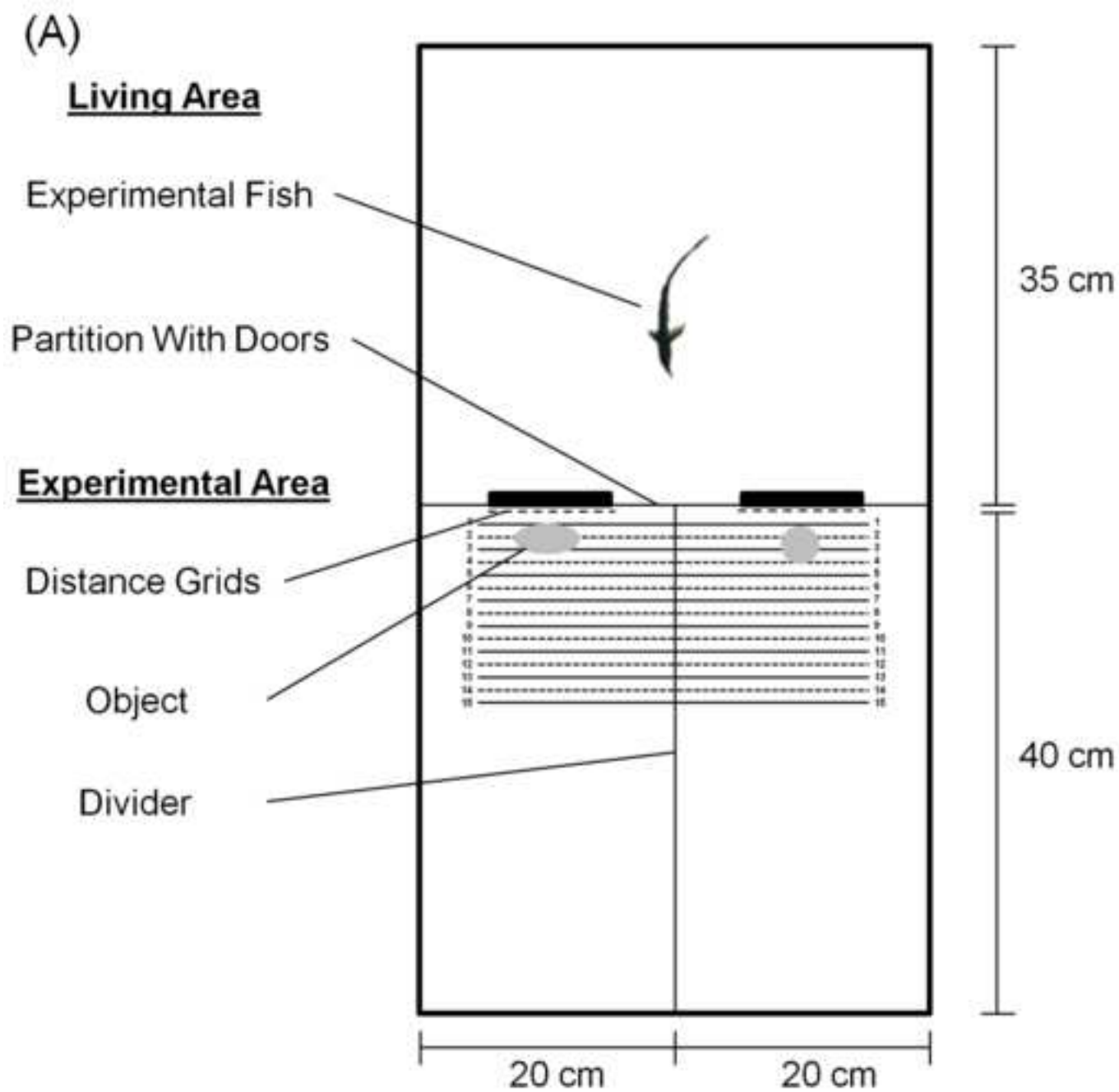
Figure 12: Relative frequency of overlapping EODs of fish 3 (blue) and fish 4 (grey) with the different playback frequencies before the trial started (A, B), during inspection of the objects (C, D) and after the trial (E, F). The left column (A, C, E) shows the results of trials, during which the fish was recorded with the playback signals present. The right column shows the results of trials, during which the fish was recorded without the playback signals present but the playback signals were added afterwards in the computer. For each point the EODs of four trials were pooled and the relative number of overlapping EODs of the experimental fish was calculated. Since the randomly expected number of overlaps depends linearly on the frequency of the playback signal, it was determined by calculating the slope of the line between the frequency, where no overlaps could occur (0 Hz) and the frequency, where 100 % of EODs must overlap (833.33 Hz). The latter was calculated by adding up the duration of the fish EOD (0.5 ms) with the duration of the playback EOD (0.7 ms) and taking the inverse of this value to receive the frequency ($1/0.012$ s).

Figure captions supplementary material:

Figure S1: Response interval histograms of fish 4 with a conspecific before the trial started (A, B), during inspection of the objects (C, D) and after the trial (E, F). For further description see Fig. 9.

Figure S2: Response interval histograms of the jamming fish 5 in response to experimental fish 4 before the trial started (A, B), during inspection of the objects (C, D) and after the trial (E, F). For further description see Fig. 9.

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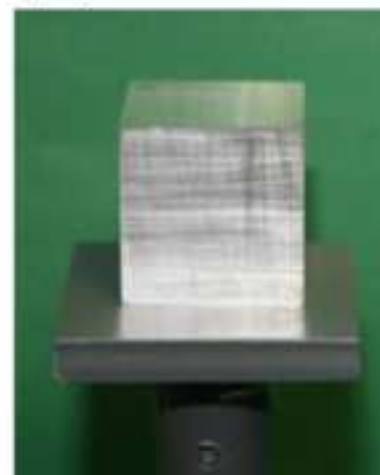
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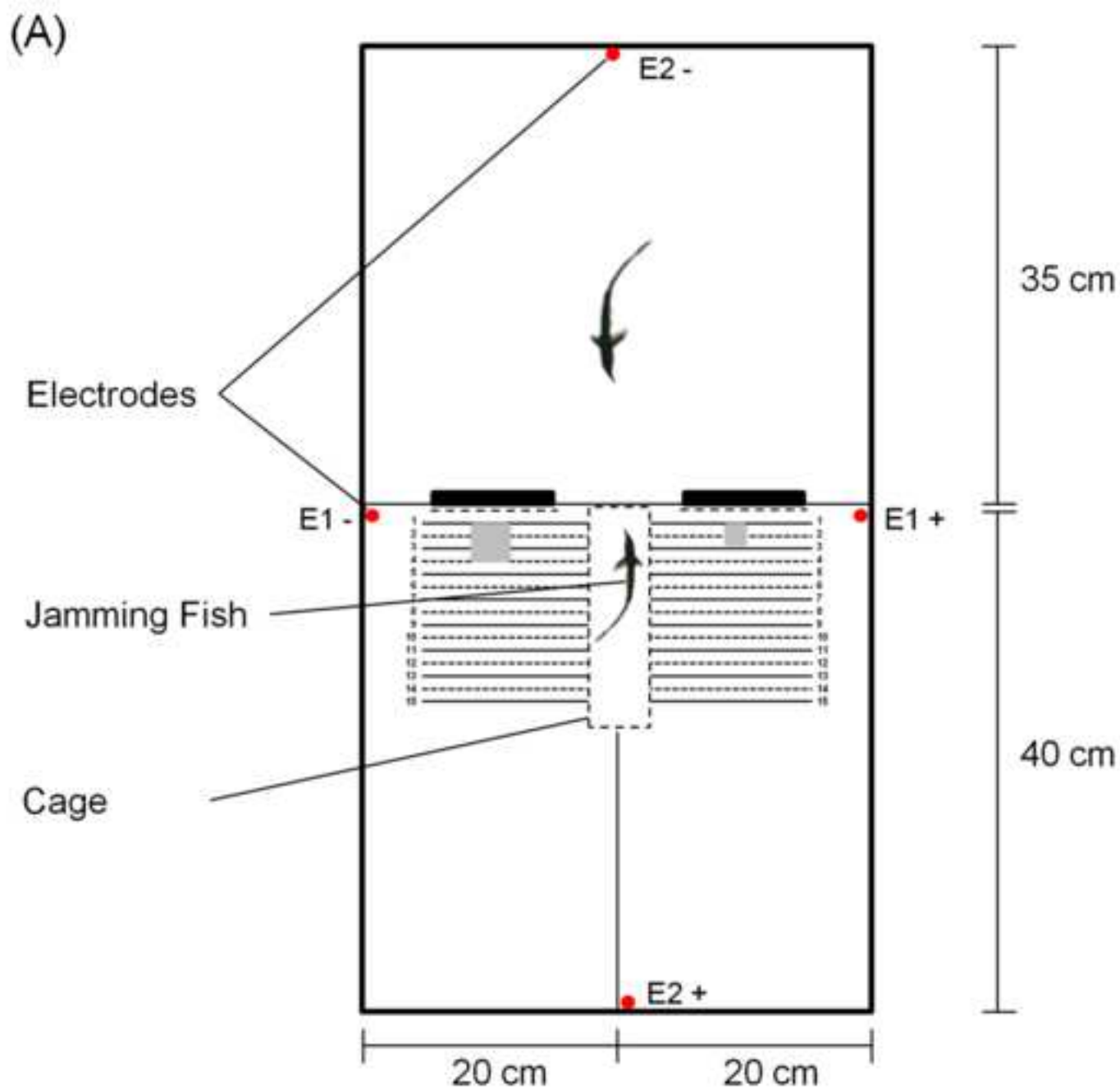


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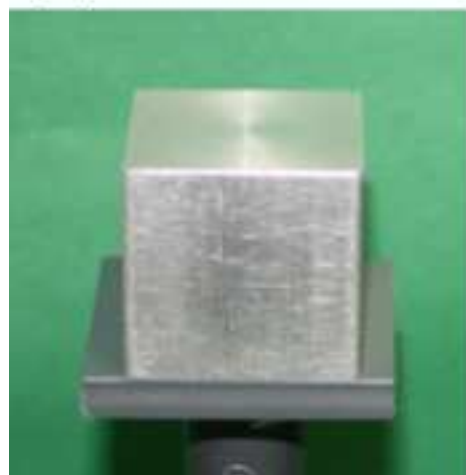


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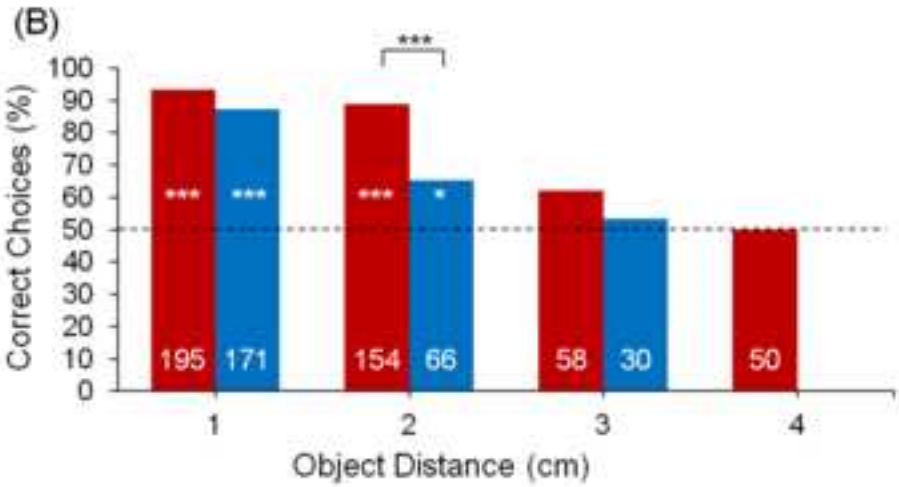
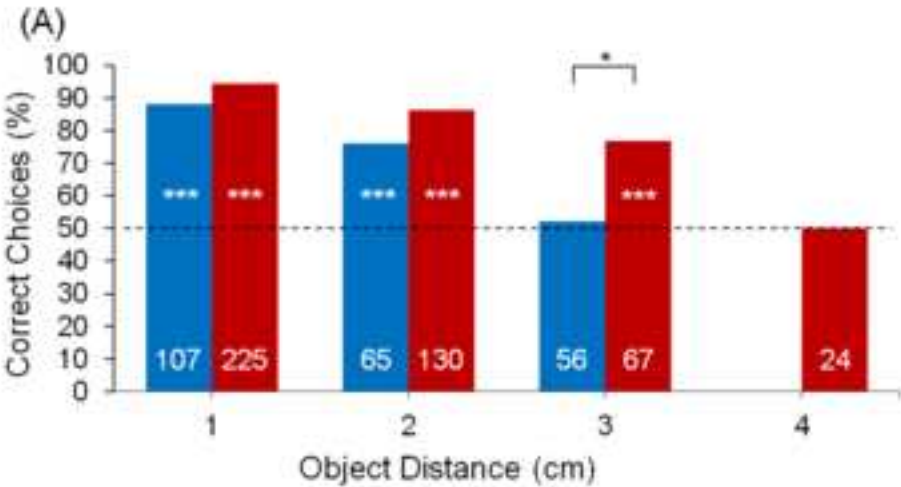


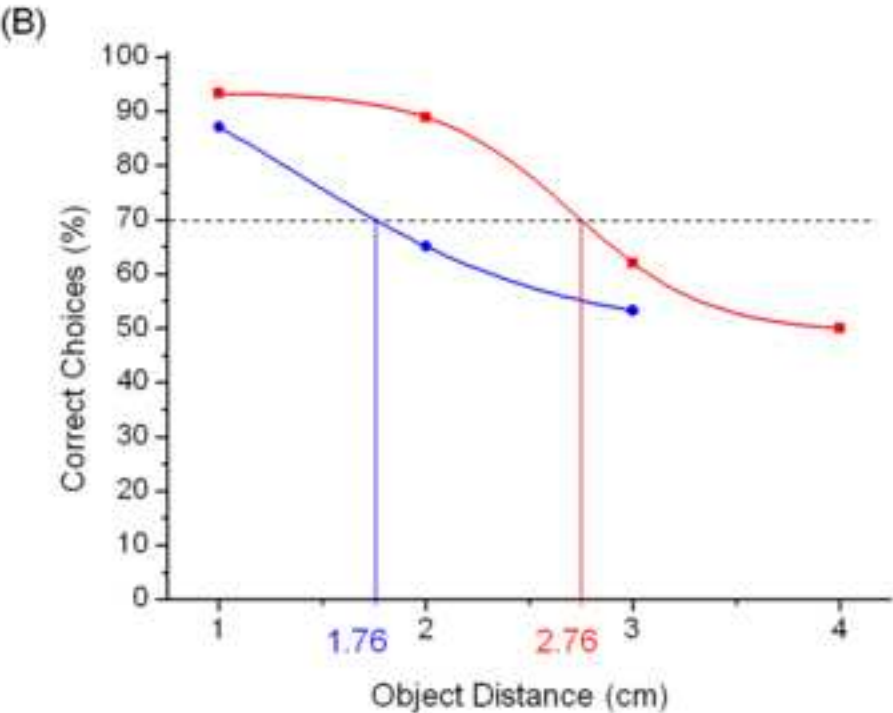
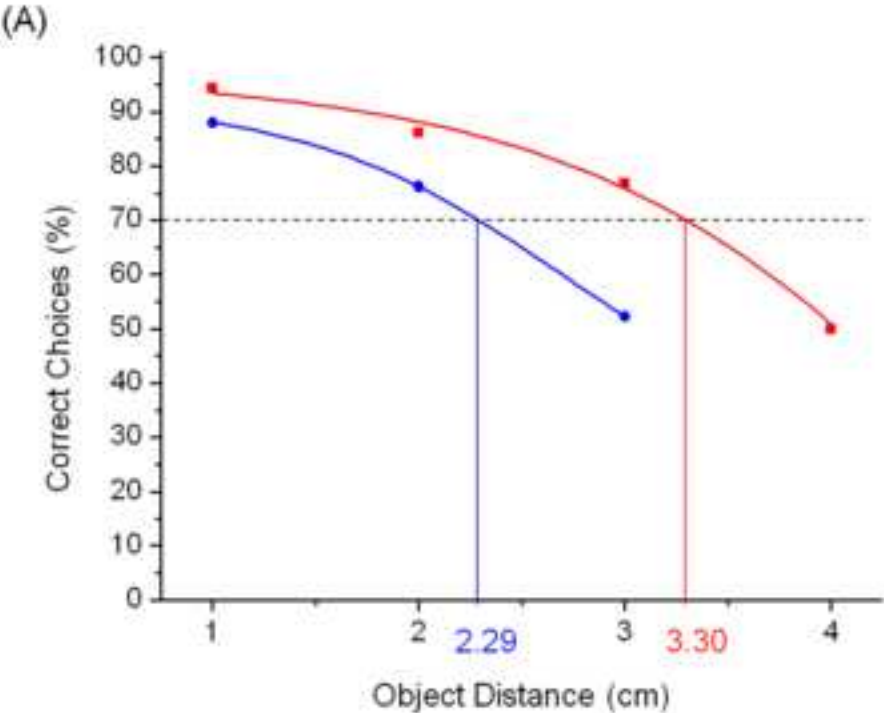
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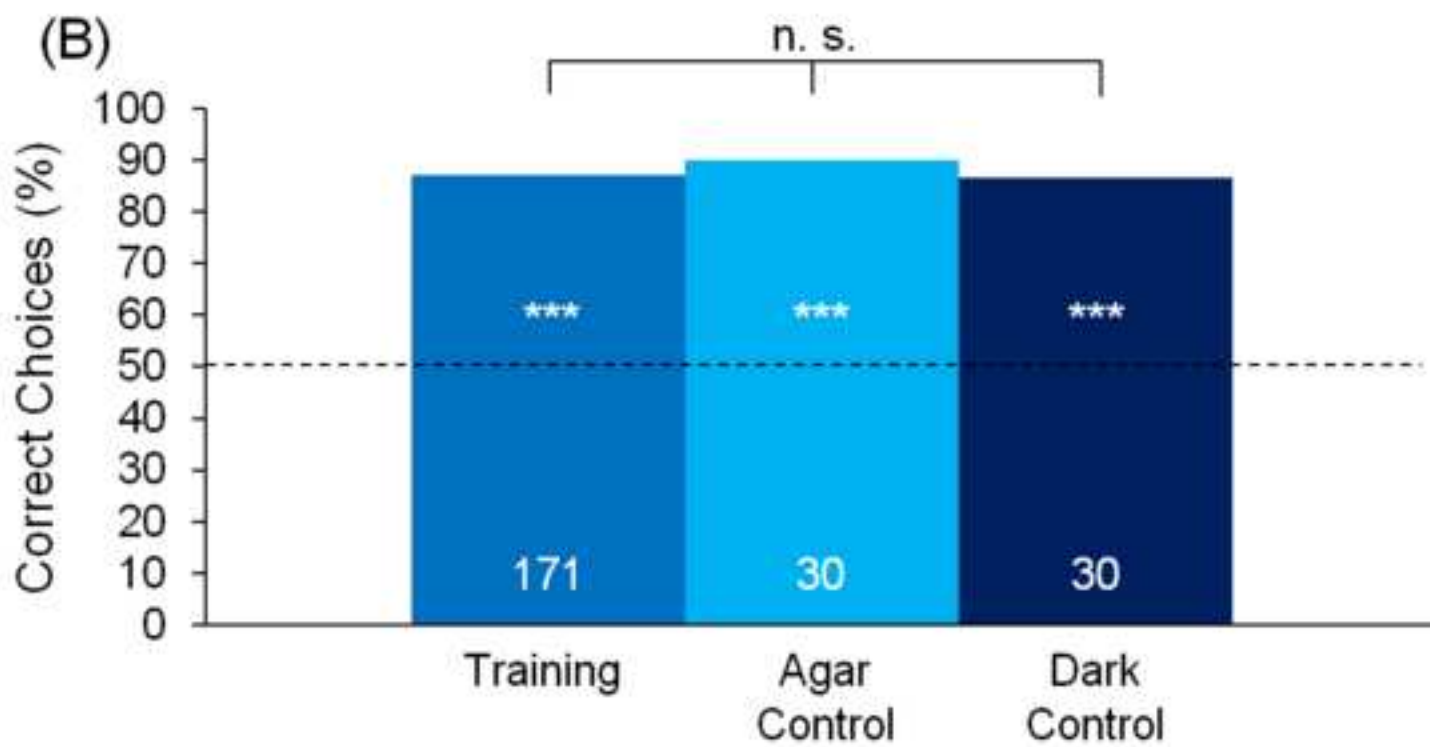
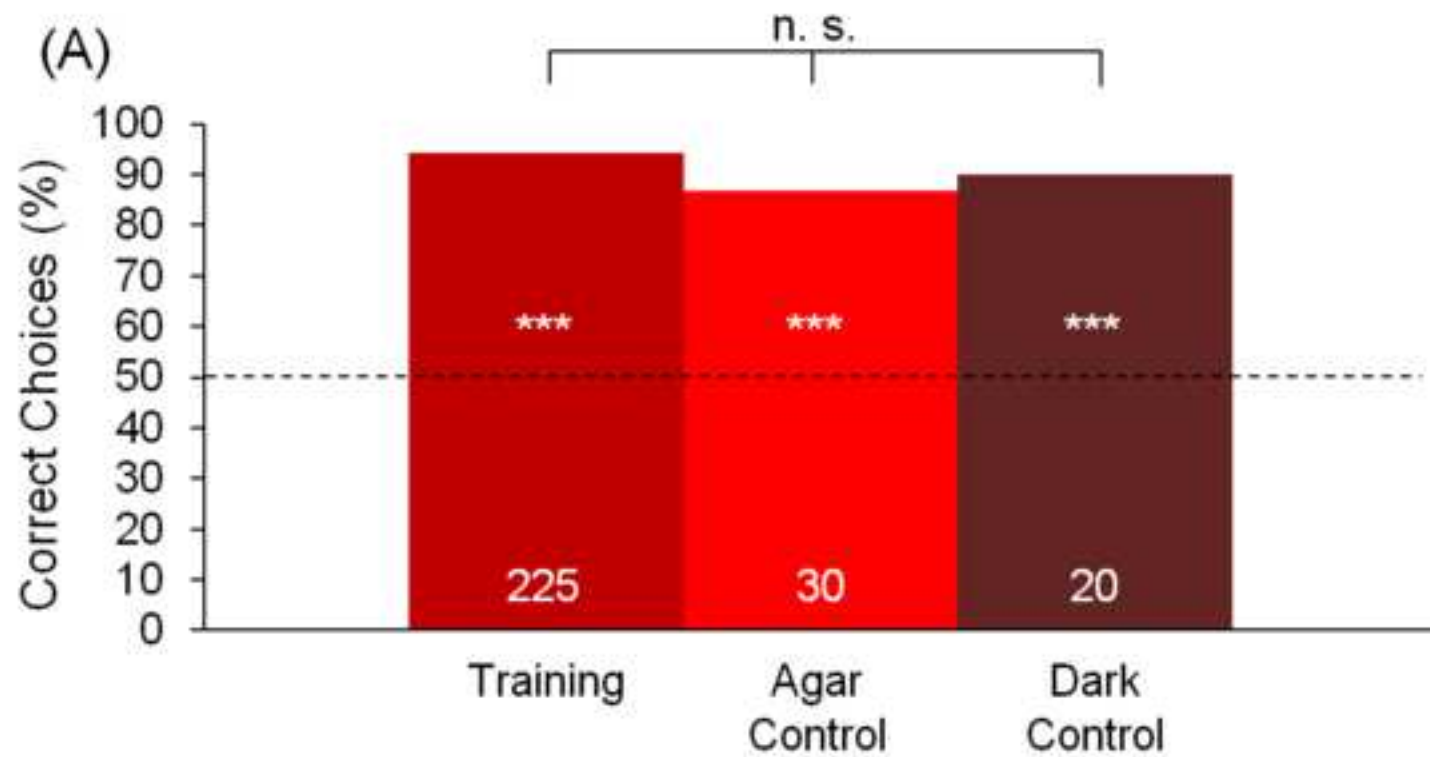


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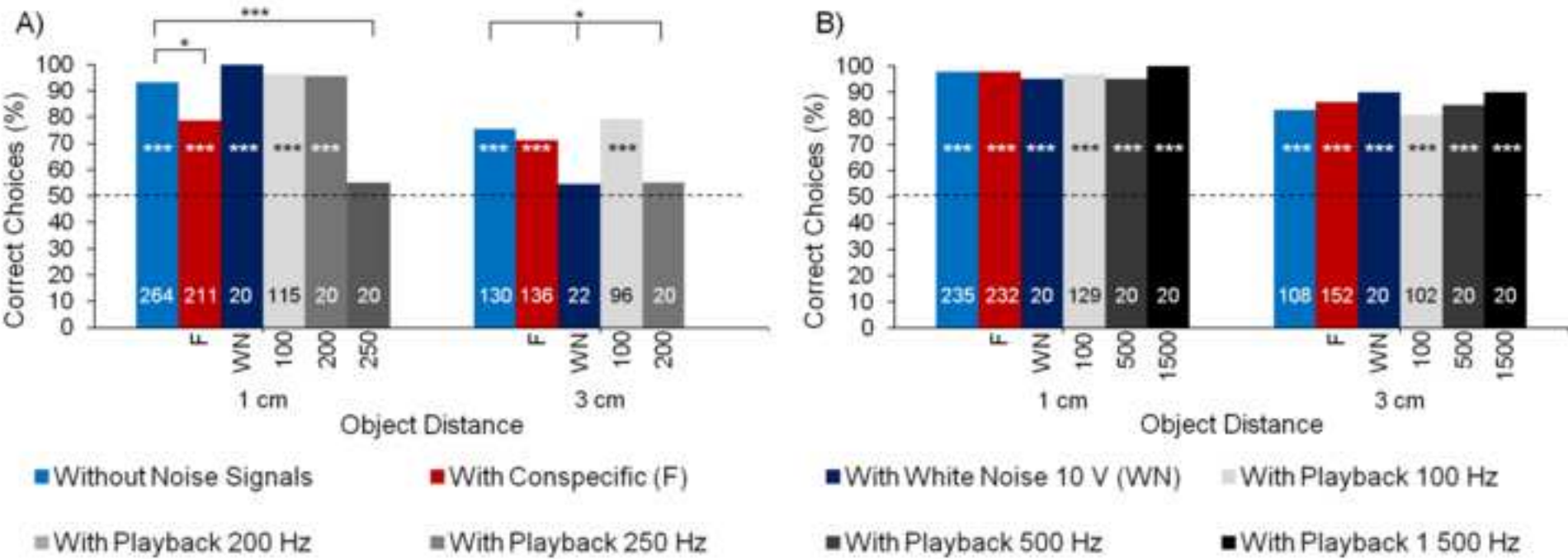


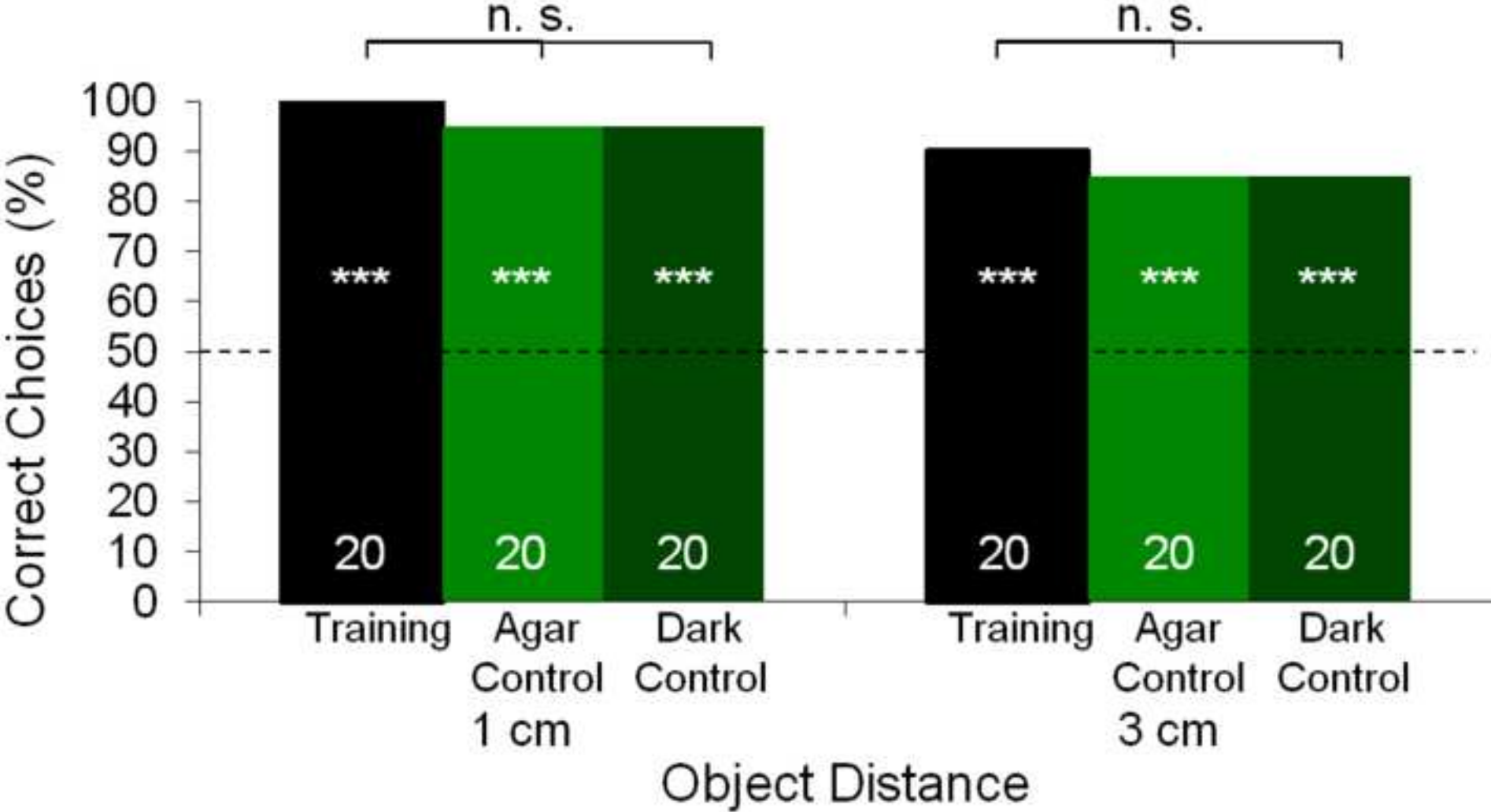




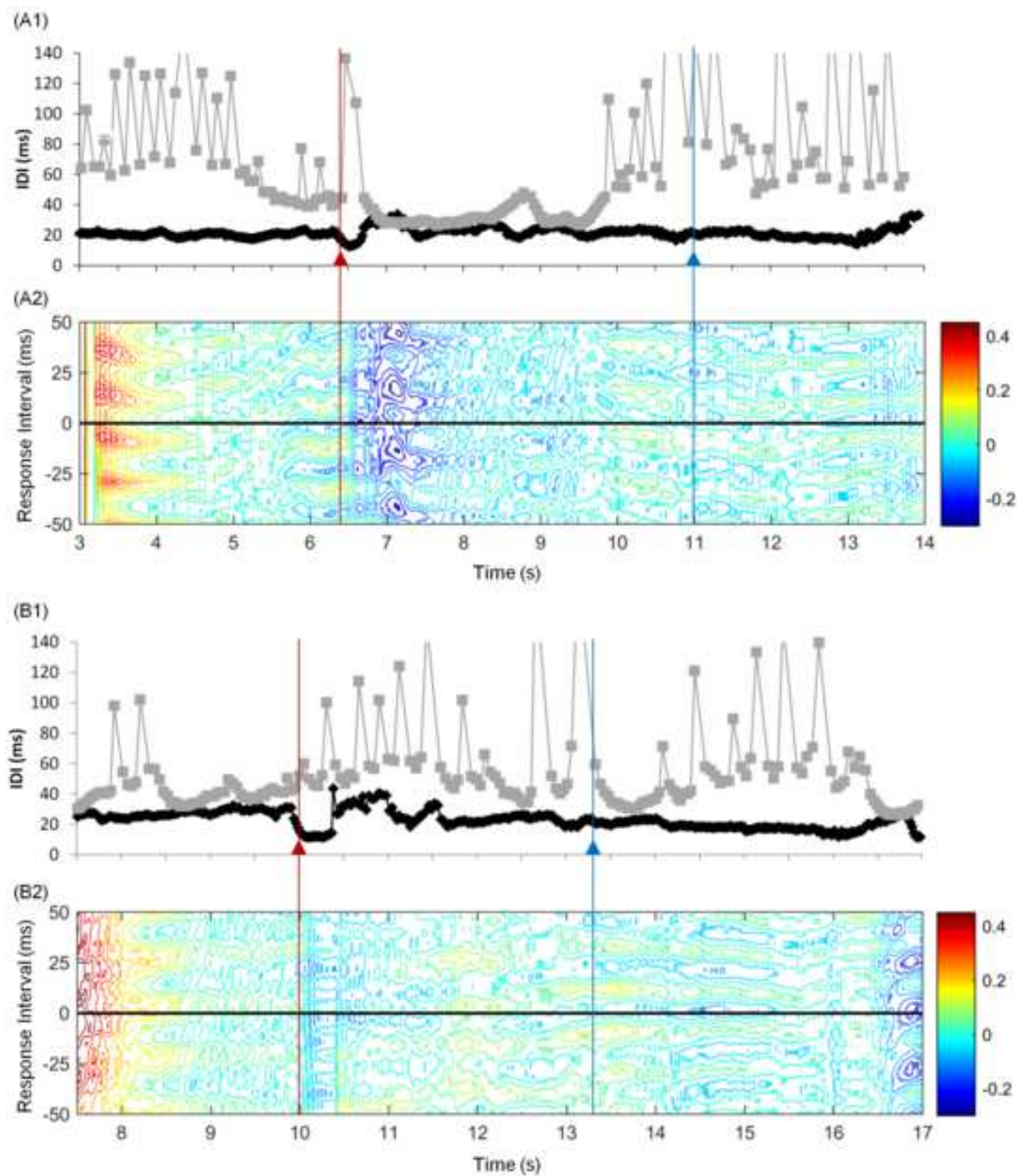


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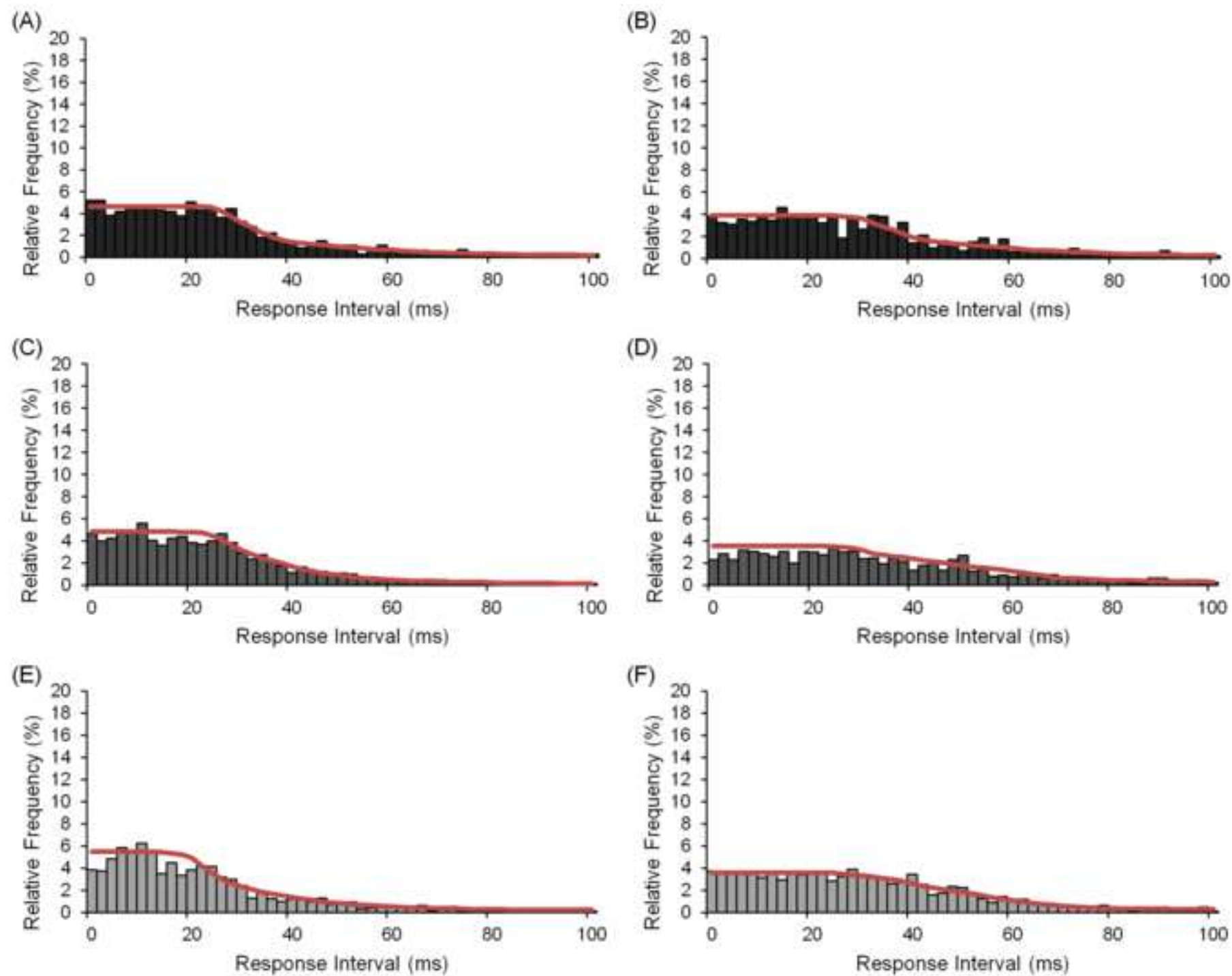


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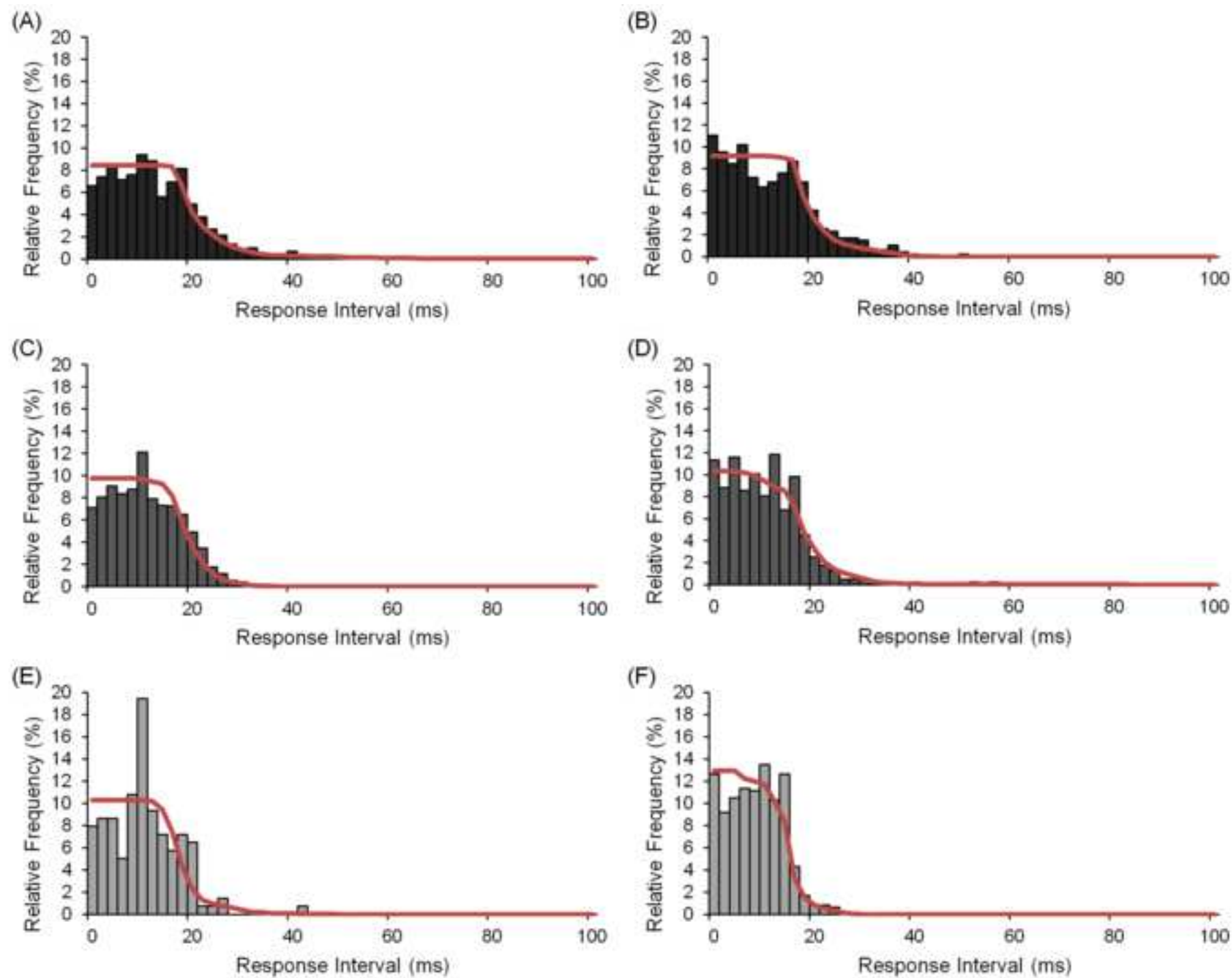
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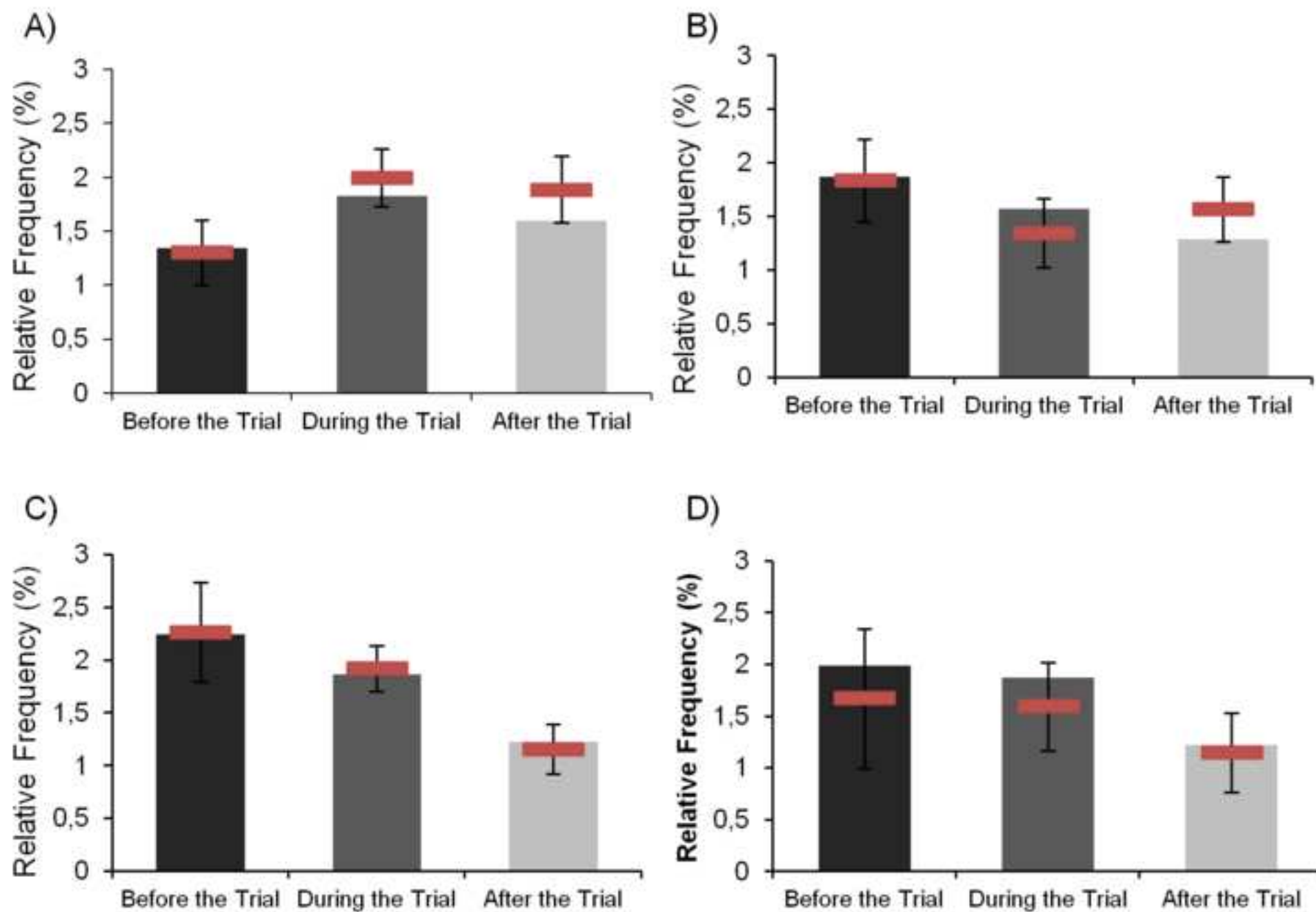


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