Science of the Total Environment 958 (2025) 178028

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Zebra_K, a kinematic analysis automated platform for assessing sensitivity, habituation and prepulse inhibition of the acoustic startle response in adult zebrafish

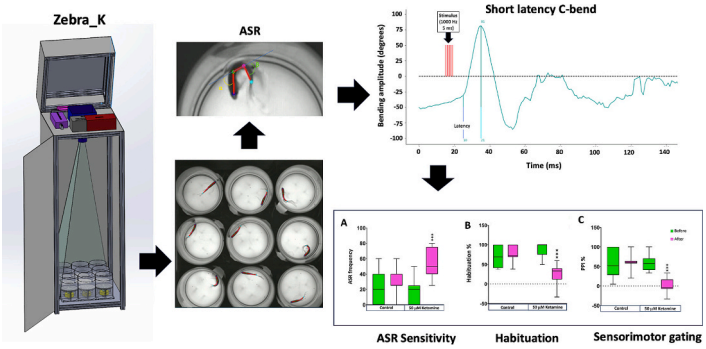
Marija Stevanović [a], Niki Tagkalidou [b], Cristiana Roberta Multisanti [c,d], Sergi Pujol [e], Ouwais Aljabasini [b], Eva Prats [f], Caterina Faggio [d,g], Josep Maria Porta [e], Carlos Barata [b], Demetrio Raldúa [b,*]

[a] Institute of Pesticides and Environmental Protection, Banatska 31b, 11080 Belgrade, Serbia
[b] Institute for Environmental Assessment and Water Research (IDAEA-CSIC), Jordi Girona, 18, 08034 Barcelona, Spain
[c] Department of Veterinary Sciences, University of Messina, Viale Giovanni Palatucci snc, 98168 Messina, Italy
[d] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Ferdinando Stagno d'Alcontres, 31, 98166 Messina, Italy
[e] Institut de Robòtica i Informàtica Industrial, CSIC-UPC, Barcelona, Spain
[f] Research and Development Center (CID-CSIC), Jordi Girona, 18, 08034 Barcelona, Spain
[g] Department of Eco-Sustainable Marine Biotechnology, Stazione Zoologica Anton Dohrn, 80122 Naples, Italy

HIGHLIGHTS

- Exposure to some neuroactive/neurotoxic pollutants impairs neuroplasticity.
- Zebra_K is an automated platform for neuroplasticity analysis in adult zebrafish.
- Zebra_K performs the kinematic analysis of the acoustic startle response (ASR).
- ASR Sensitivity, habituation and prepulse inhibition are determined by Zebra_K.
- NMDA-receptor antagonists, gender and time of day modulate ASR plasticity.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Henner Hollert

Keywords:
Acoustic startle response
Neuroplasticity
Habituation
Prepulse inhibition
Sensorimotor gating

ABSTRACT

The acoustic startle response (ASR) is leaded by a sudden and intense acoustic stimulus. ASR has several forms of plasticity, including habituation and sensorimotor gating. Although ASR and its plasticity have been intensively studied in zebrafish (*Danio rerio*) larvae, information in adult zebrafish is still very scarce. In this manuscript we present Zebra_K, a new automated high-content kinematic analysis platform for assessing ASR, its habituation and prepulse inhibition (PPI), a quantitative measure of sensorimotor gating, in adult zebrafish. The analysis of the kinematic parameters of ASR in adult zebrafish has shown a single response wave consistent with the short-latency C-bend described in zebrafish larvae. Moreover, protocols have been designed and validated in Zebra_K for the analysis of sensitivity, habituation and PPI of this response. Then, the effect of the time of day and the

* Corresponding author.
E-mail address: drpqam@cid.csic.es (D. Raldúa).

https://doi.org/10.1016/j.scitotenv.2024.178028
Received 18 September 2024; Received in revised form 18 November 2024; Accepted 7 December 2024
Available online 15 December 2024

Adult zebrafish
Kinematic analysis platform

gender on zebrafish ASR plasticity has been analyzed for the first time. Females exhibited higher responsiveness and a lower habituation and PPI than males, a result consistent with the gender effect described in other animal models and in humans. This platform has also been used to determine the effect of a pharmacological modulators of ASR plasticity, the NMDA-receptor antagonist ketamine. As described in other animal models, ketamine increased the responsiveness to the acoustic stimuli, decreasing habituation and leading to complete abolition of PPI. These results enhance the interest of using adult zebrafish to assess the potential effect of environmental pollutants on ASR plasticity.

1. Introduction

The startle reflex is a fast motor response triggered by a sudden and intense stimulus, probably aiming to protect the animals against injury from a potential hazard. In the acoustic startle response (ASR), the reflex is evoked by an acoustic/vibrational stimulus (Lauer et al., 2017). The basic ASR circuit begins when the cochlear hair cells are activated by a loud sound (>80 dB in mammals). The information is transmitted to the cochlear root neurons (CRNs) through the auditory nerve and, once excited, the CRNs sends excitatory inputs to the caudal pontine reticular nucleus (PnC). Activation of PnC leads to the activation of cranial and spinal motoneurons and, finally, to the motor response, eyelid closure and contraction of facial, neck and skeletal muscles in mammals (Gómez-Nieto et al., 2014). ASR is characterized by a very short latency and duration. Abnormal reactivity of ASR has been found in mammalian models of different neuropsychiatric conditions such as post-traumatic stress disorder (PTSD), Fragile-X syndrome, or Alzheimer's disease (Chen and Toth, 2001; O'Leary et al., 2017; Siegelaar et al., 2006).

ASR exhibits several forms of behavioral plasticity, including habituation and prepulse inhibition (PPI), and is therefore commonly used to screen the effects of drugs on sensorimotor gating in different animal models (Gómez-Nieto et al., 2020; Koch, 1999; Lauer et al., 2017; López-Schier, 2019). Habituation is a neuroplastic process, considered as a non-associative form of learning, in which an organism "learns" to filter out irrelevant stimuli (López-Schier, 2019). Short-term habituation to the ASR occurs within a single session using a short interstimulus interval (ISI). In PPI, the magnitude of ASR is reduced when a non-startling stimulus (prepulse) is presented 30–500 ms before the startling stimulus (pulse) (Swerdlow and Geyer, 1998). PPI is used as a quantitative measure of sensorimotor gating (Gómez-Nieto et al., 2020). Both habituation and PPI are considered filtering mechanisms of the central nervous system (CNS) to prevent information overload, allowing selective attention and right processing of the information (Gómez-Nieto et al., 2020). ASR neuroplasticity processes are clinically relevant as they are affected in various neuropsychiatric disorders, including schizophrenia, Fragile-X syndrome, obsessive-compulsive disorder (Hoenig et al., 2005; López-Schier, 2019; Sato, 2020; Swerdlow and Geyer, 1998), neurodegenerative disorders, such as Parkinson's, Alzheimer's or Huntington's diseases (Jafari et al., 2020; Leng et al., 2004), as well as after the consumption of some recreational drugs such as MDMA or cocaine (Preller et al., 2013; Quednow et al., 2004).

Zebrafish (*Danio rerio*) is a vertebrate experimental model increasingly used in the neurobiology field for several factors, including its high degree of similarity to mammalian models and humans in both the overall organization of the nervous and neurotransmitter systems (Babin et al., 2014). Behavioral repertoire in zebrafish is rich, including anxiety-like behavior, social behavior, learning and different types of memory, to name but a few (López-Schier, 2019). In addition, the genes involved in neurodegenerative diseases are also highly conserved (Babin et al., 2014). Like mammals, zebrafish display a robust ASR in response to acoustic/vibrational stimuli (Fero et al., 2011). Zebrafish ASR is mediated by the Mauthner-cell neuronal circuit, in which Mauthner cells receive sensory inputs from the acoustic-lateralis and vestibular systems (Medan and Preuss, 2014). A single action potential in one of the Mauthner cells is sufficient to trigger the motor networks in the contralateral trunk muscle, simultaneously inhibiting those on the ipsilateral side (Medan and Preuss, 2014). The kinematic analysis of zebrafish ASR has shown that it is characterized by a C-bend of the body, followed by a smaller counter-bend, and then a fast-swimming bout (Fero et al., 2011). Like in mammals, zebrafish ASR also has a very short latency and duration (Burgess and Granato, 2007a). Zebrafish ASR is considered a model system to study startle plasticity, including habituation and PPI (Burgess and Granato, 2007a; Medan and Preuss, 2014; Wolman et al., 2011). Although ASR is displayed by larval and adult zebrafish (Burgess and Granato, 2007a), most of the available information is restricted to larvae. Different platforms for assessing ASR kinematics in zebrafish larvae have been developed by research groups studying startle plasticity (Burgess and Granato, 2007a; Wolman et al., 2011), and a platform for assessing PPI is commercially available (ZebraBox PPI Hybrid, from ViewPoint (Banono and Esguerra, 2020)). These technological tools have allowed the high-content screening of small molecules able to modulate ASR and its plasticity in zebrafish larvae (Wolman et al., 2011). However, to our knowledge, no similar platforms have been developed to assess ASR plasticity in adult zebrafish, making it difficult to analyze the long-term effects of developmental exposures to neurotoxic compounds, the study of late-onset neurodegenerative diseases or the effect of the gender on these processes, to give just a few examples.

In this manuscript we have developed a new automated kinematic analysis platform to assess the habituation and PPI of acoustic startle in nine adult zebrafish simultaneously. The main kinematic parameters of the initial C-bend have been characterized using different types of acoustic stimuli. Then, the effect of the time of day and the gender on these ASR neuroplasticity processes has been explored. Finally, conditions for habituation and PPI analysis have been further standardized, and the reliability of the platform has been tested by using ketamine, a non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist.

2. Material and methods

2.1. Animals and housing

Adult wild-type zebrafish (standard length: 2.0–3.0 cm) were obtained from Pisciber (Terrassa, Spain) and maintained into a recirculating zebrafish system (Aquaneering Inc., San Diego, United States) at the Research and Development Center zebrafish facilities (CID-CSIC) for 2 months before starting the exposures. Fish were housed in 2.8 L tanks (density: 20 fish/tank) with fish water [reverse-osmosis purified water containing 90 mg/L Instant Ocean® (Aquarium Systems, Sarrebourg, France), 0.58 mM $CaSO_4 \cdot 2H_2O$, and 0.59 mM $NaHCO_3$] under a 12 L:12D photoperiod. The main parameters of the fish water in the housing facilities were: temperature: 28 ± 1 °C; pH: 7.6–8.0; conductivity: 700–800 μS/cm; hardness: 120–130 mg/L. Fish were fed twice a day with flake food (TetraMin, Tetra, Germany).

All procedures were approved by the Institutional Animal Care and Use Committees at the CID-CSIC (OH 1032/2020) and conducted in accordance with the institutional guidelines under a license from the local government (agreement number 11336). Importantly, the ASR analysis and its associated plasticity processes are conducted using non-invasive procedures that do not cause any lasting harm to the animals. Except for the fish exposed to ketamine, all other fish involved in the assays exhibited normal behavior and showed no signs of distress after

the completion of the experiments. Therefore, with the sole exception of those fish used for the ketamine tests, which were euthanized after completion of the experiments, all other fish used for this study were returned to the animal facility and used as breeders for future research in accordance with ethical guidelines and institutional policies.

2.2. Zebra_K observation chamber

The Zebra_K observation chamber (Fig. 1A-B) was designed and built at the Institut of Robòtica i Informàtica Industrial (CSIC-UPC). A schema of the principal components and connections of this device is shown in Fig. 1C. The setup includes nine cylindrical glass tanks (diameter 73 cm, height 60 cm), each holding a single adult zebrafish in 40 mL of fish water. The tanks are organized in a 3 × 3 grid and they are placed in a closed chamber to isolated them from the vibrations and illumination in the environment. As show in Fig. 1D, each tank is placed on the top of a translucent surface providing infrared illumination using 16 LEDs (OSRAM SFH 4556) regularly distributed in the base of the tank. A speaker of 66 mm of diameter, 4 Ohm and 5 W (Amazon, ASIN: B097BHFDSX) is placed under the illumination system, at 60 mm of the base of the tank. This speaker is used to generate vibrations that are transmitted to the tank bypassing the illumination system. The sound pressure level (SPL) of the stimuli on the tanks provided by the speakers was determined by a sonometer PCE-322A (PCE Instruments, Meschede, Germany). To capture the reactions of the fish to the vibrations, we installed a high-speed camera (Photron Fastcam Mini UX100) equipped with a 28 mm lens (Sigma 28 mm F1.4 DG HSM Art) on top of the device. When active, the camera takes 1024 × 1024 pixel images at 1000 frames per second. The camera trigger is activated by a microprocessor (Arm Cortex-M4-based STM32F4) which is also responsible for generating the acoustic stimuli. In this way, a precise synchronization between the stimuli and the captured videos is achieved.

2.3. Zebra_K: software description

Two software packages are provided to properly operate the device, one to control the experiments and one to analyze the images obtained in each experiment. Both can be run on a standard PC. The ZK Control Software takes care of generating the acoustic stimuli and capturing the images with the potential fish reactions to such stimuli. The main window of this software is shown in Supplementary Fig. S1. This is a highly-flexible software that allows the generation of a wide variety of sequences of acoustic stimuli patterns with arbitrary pauses between them and with an arbitrary number of repetitions for each pattern sequence. Moreover, the intensity and duration of each stimulus can be set individually. The software takes care of translating the configured experiment into low-level commands that are sent to the microcontroller via a USB connection. Finally, this same software is also responsible for downloading the videos captured by the high-speed camera, which is connected to the PC via a gigabit ethernet cable.

The videos captured with the control software are processed with Zebrafish Acoustic Startle Response kinematic analysis software (Supplementary Fig. S2). This software examines the video frame by frame. For each frame, it locates the nine tanks and then, for each tank, it determines the posture of the corresponding fish (Supplementary Fig. S3). To this end, four relevant points on the fish are identified, namely, the head, a point on the upper body, another one on the lower body, and the tail. These four points, robustly identified using a deep neural network trained with DeepLabCut (Lauer et al., 2022; Mathis et al., 2018; Nath et al., 2019), are used to characterize the posture of the fish using the angles shown in Fig. 2A-B. More specifically, the computed angles are the one between the first and second segment (α), the one between the second and third segment (β), and the sum of both (γ). The temporal evolution of γ gives an accurate account of the reaction of the fish to the stimuli. The microprocessor and the high-speed camera allow the identification of the reactions with an accuracy up to the millisecond. In this way, all fish reactions can be properly detected. To facilitate the data analysis of each experiment, the software generates a comprehensive report with graphs of the analyzed angles, automatically identifying the escape reaction event (Fig. 2C). Finally, the software summarizes the relevant statistics of the entire experiment, and outputs a report including those parameters defined in Fig. 2D. These are the data finally used to evaluate the fish responses.

2.4. Experimental procedure

All the experiments with Zebra_K were performed in an isolated behavioral room at 27–28 °C, between 10:00–17:00 h. Fish were acclimated to the behavior room 1 h before starting the experiments.

To characterize the kinematic endpoints of ASR in adult zebrafish, a strong intensity acoustic/vibrational (AV) stimulus (startle-inducing stimulus) inducing 60–80 % startle responses in short-fin wild-type adult zebrafish was used (Burgess and Granato, 2007a).

The protocol used for short-term habituation studies, based on that described by Wolman et al. (2011) for zebrafish larvae, includes 4 steps. First, during the sensitivity step, adults were exposed to 5 moderate-level AV stimuli, typically eliciting 15–30 % of startle responses, delivered with an interstimulus interval (ISI) of 120 s. Then, during the prehabituation step, 5 startle-inducing AV stimuli were delivered with an ISI of 120 s. During the habituation step, 15 startle-inducing AV stimuli were delivered with an ISI of only 1 s. Finally, after 300 s of resting time, the recovery step was characterized by 5 startle-inducing AV stimuli delivered every 120 s. The degree of habituation was calculated as the ratio between the average startle responses during the last 5 stimuli of the habituation step and the 5 stimuli of the prehabituation step (Wolman et al., 2011).

For PPI analysis, a low-intensity AV stimuli, typically eliciting 0–10 % startle responses, was selected as the prepulse stimulus, and the startle-inducing AV stimulus was selected for the pulse. A series of 5 low-intensity AV stimuli (IS: 120 s), then a series of 5 startle-inducing AV stimuli (ISI: 120 s), and finally, a series of 5 sequences "prepulse+pulse" AV stimuli (ISI: 120 s) were delivered. For these sequences, the effect of different times between the prepulse and the pulse, from 1 ms to 2 s, on PPI was tested. The PPI percentage was calculated as described elsewhere (Burgess and Granato, 2007a):

2.5. Effect of time of day

To assess the effect of time of day on the responsiveness and habituation, these parameters were analyzed at 10:00, 14:00, 16:00, and 18:00, a period of time when behavioral experiments could be performed. At each time point, 8 male adult fish were located to the Zebra_K and sensitivity, prehabituation and habituation were determined. Only experimentally naïf fish were used at each time point. For each time point, 2 (16:00) or 3 (10:00, 14:00, and 18:00) replicates were obtained in different days. A similar design was followed to determine the effect of time of day on the percentage of PPI.

2.6. Effect of gender on ASR plasticity

To assess the effect of the gender on sensitivity and habituation, 24 male and 24 female adult fish were distributed into 6 experimental groups. The standard length of the selected males and females is shown in Supplementary Table S1. The experiments were conducted in 2 different days, with three trials per day. A similar design was followed to determine the effect of gender on the percentage of PPI.

2.7. Pharmacological modulation of ASR

Ketamine hydrochloride (K2763; Purity = 100 %) was provided by Sigma-Aldrich (St. Louis, Missouri, USA). To investigate the effect of ketamine on sensitivity, habituation, and PPI, a pretest-posttest control

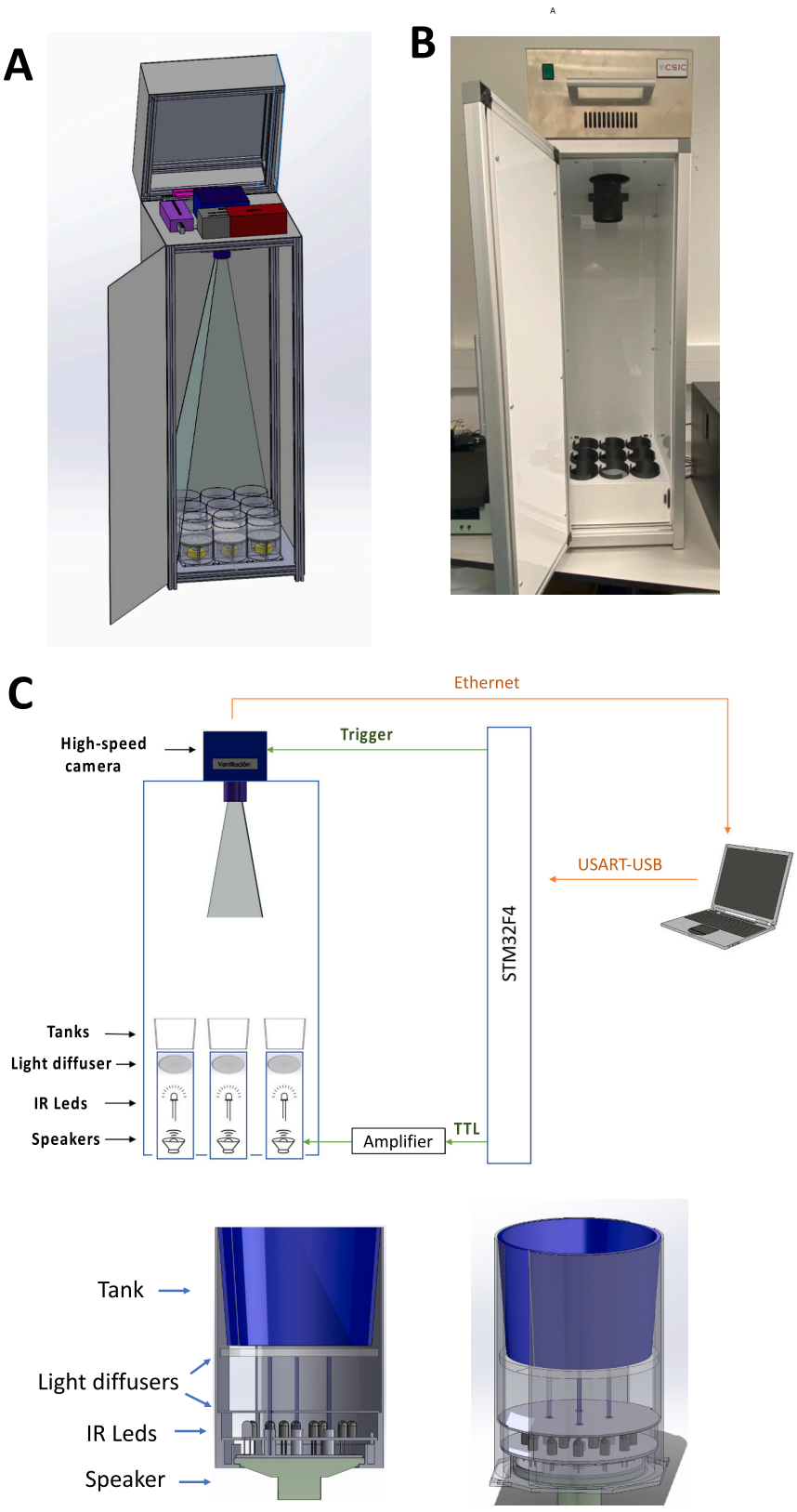
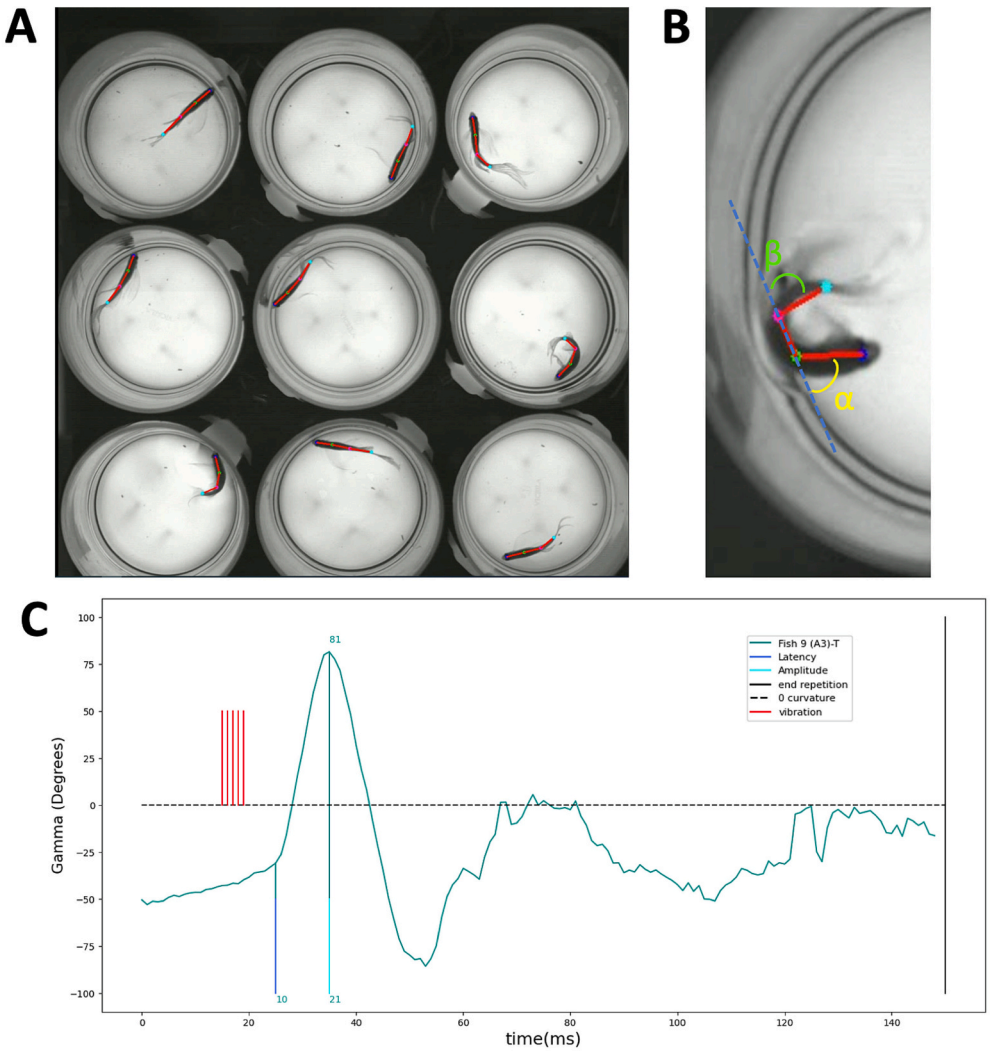


Fig. 1. Zebra_K observation chamber. (A-B) General view of the experimental Zebra_K observation chamber, including the initial model (A) and its actual implementation (B); (C) Diagram of the platform, identifying the main components and connections of the observation chamber; (D) Detail of the illumination and vibration system for each tank.



D

Parameter	Formula	Description
Gamma (γ_t)	$\gamma_t = \alpha_t + \beta_t$	Curvature of the fish taking at time t (in ms) into account both the head and the tail angles
Linear velocity (v_t)	$v_t = \frac{p_{t-1} - p_t}{\Delta_t}$	Velocity between frames using the fish head point coordinates. Δ_t is the time between frames
γ_t velocity (ω_t)	$\omega_t = \frac{\gamma_{t-1} - \gamma_t}{\Delta_t}$	Velocity of γ at time t
Latency (t_λ)	First t such that $\omega_t > thresh$	Time where the fish reaction is detected after the beginning of the vibration. We assume that the fish reacts when ω_t surpasses a fixed threshold
Amplitude (t_α)	t such that γ_t is maximum for t in $[t_\lambda, t_f]$	Time where γ_t is maximum during the scape reaction (i.e. between t_λ and t_f, where t_f is the time at the end of the reaction)
Max γ velocity	$\max(\omega_t \mid t \in [t_\lambda, t_f])$	Maximum ω_t between t_λ and t_α
Bending (b)	$b = \gamma_{t_\alpha} - \gamma_{t_\lambda}$	Difference in the γ at t_α and at t_λ

Fig. 2. Zebrafish Acoustic Startle Response Kinematic Analysis. (A) Zebrafish in the 3 × 3 grid, as captured by the high-speed camera. The red line marks its dorsal and the three segments delimited by the four colored dots approximate its posture, characterized by the angles shown in B. (B) Capture of a zebrafish skeleton, α and β angles provide the curvature of the head and the tail respectively. (C) Characterization of the adult zebrafish escape response through the kinematic analysis of the body axis curvature ($\gamma\gamma$). (D) Definition of the main parameters reported by the Zebrafish Acoustic Startle Response Kinematic Analysis Software.

group design (Dimitrov and Rumrill, 2003) approach was used. This design allows for the comparison of responses within and between control and treated groups before and after ketamine administration. A total of 56 adult fish were used in the pharmacological study. Fish were randomly assigned to either the control group ($n = 28$) or the ketamine-treated group ($n = 26$). Half of the fish in each group were used for habituation experiments (control: $n = 14$, ketamine-treated: $n = 13$), while the remaining half were used for PPI experiments (control: n = 14, ketamine-treated: n = 13). The standard length of fish in each group is shown in Supplementary Table S1.

Baseline measurements of ASR sensitivity, habituation, and PPI were obtained in the Zebra_K for all fish. Then, the ketamine-assigned group was transferred to a new tank containing a 50 μM ketamine solution, while the control group was transferred to a new tank with fish water. Post-treatment measurements of ASR, habituation, and PPI were conducted 20 min after exposure. All three endpoints were calculated for each fish both before and after the treatment and the changes observed from pre-test to post-test were analyzed.

2.8. Reliability analysis

For measuring the accuracy, the latency, maximum bending and duration of the bending were analyzed manually in fifty videos containing ASRs. For the calculation of the maximal bending, angles α and β during each reaction were analyzed frame by frame using the free graphical image analysis software ImageJ (National Institute of Health, Bethesda, MD, USA). Moreover, the robustness and consistency of the responses were measured across experiments. Robustness was assessed by determining the variability between groups of fishes within and between experiments conducted in the same or in different days. On same days, the same group of fish was assayed across experiments, thus it was possible to account for variability across and within different fish groups, hereafter referred to as group or fish, respectively. We have used the responses in control treatments across the parameters shown in Supplementary Table S2. In the two ANOVA model designs used, group or fish were considered nested and random factor and day as a fixed one. Variance component depicted as % of total variance of those analyses were also quantify using the MINQUE (minimum norm quadratic unbiased estimator) produces. Prior to analyses data was tested for normality and, if necessary, arccosine transformed.

2.9. Statistical analysis

Data were analyzed with IBM SPSS v29 (Statistical Package 2010, Chicago, IL, USA) and plotted with GraphPad Prism 9 for Windows (GraphPad software Inc., La Jolla, CA, USA).

In order to determine if the samples followed a normal distribution, a Shapiro–Wilk test was used. Descriptive statistics were presented as mean ± standard error (SEM) for parametric data, and as median and interquartile range (IQR) for non-parametric data. For normally distributed groups, an unpaired *t*-test was used to determine statistical significance and one-way ANOVA followed by Dunnett's as a multiple comparison test. When parametric assumptions could not be made, statistical significance was determined by a Mann–Whitney *U* test and a Kruskal–Wallis test followed by Dunn–Bonferroni's test to see if there were any differences between more than two groups. Significance was set at $p < 0.05$.

3. Results and discussion

3.1. Kinematic parameters of the acoustic startle response in adult zebrafish

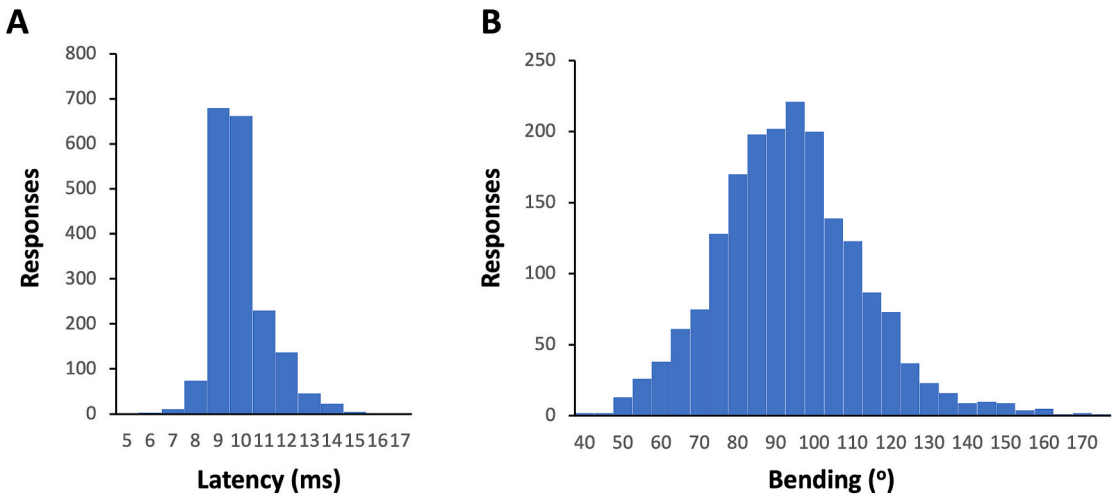
To systematically determine the kinematic parameters of ASR in adult zebrafish, Zebra_K, an automated kinematic analysis platform able to assess the acoustic startle in adult zebrafish, has been developed. The Zebra_K Observation Chamber (Fig. 1) includes nine cylindrical tanks, organized in a 3 × 3 grid, and the acoustic stimuli are provided by speakers located under each tank. Illumination is provided by 16 near-infrared leds under each tank, and the fish reactions are recorded at 1000 frames per second (fps) using a high-speed camera located on top of the chamber. The Zebra_K control software allows to determine the number, frequency, duration and intensity of the acoustic stimuli, as well as the interstimuli intervals. Finally, four points on each fish are identified using the Zebrafish ASR kinematic analysis software, a trained deep neural network, and different kinematic parameters are determined, including the latency and the maximum angular velocity, duration and amplitude of the C-bend.

As shown in Fig. 3, kinematic analysis of 1875 ASRs performed by wild-type short-fin adult zebrafish in response to a startle-inducing stimulus (1000 Hz/ 1 ms/ 103.9 dB re 20 μPa) showed only one wave of responses, characterized by a short latency [median (IQR): 10.0 ms (9–11 ms)] and duration [10.0 ms (9–12 ms)] (Supplementary Dataset 1). These kinematic values found in the ASR of adult fish are consistent with those previously reported for the short-latency C-bend (SLC) in zebrafish larvae (Brun et al., 2021; Burgess and Granato, 2007a; Meserve et al., 2021; Panlilio et al., 2020; Santistevan et al., 2022; Scaramella et al., 2022). Moreover, in contrast to the ASR reported in larvae (Burgess and Granato, 2007a), no evidence has been found in this study of long latency C-bend (LLC) in adult zebrafish exposed to startle stimuli. Burgess and Granato (2007a) also found an ASR consistent with SLC in adult zebrafish (TLF wild-type line), and furthermore, they found no evidences of LLC in the behavioral repertoire of the adult fish.

3.2. Short-term habituation of ASR in adult zebrafish

To assess the short-term habituation of ASR, the four-step protocol designed by Wolman et al. (2011) for larvae has been adapted for adult zebrafish (Fig. 4A). Each step is based in delivering a series of stimuli, with moderate or high intensity level depending on the step, separated by specific interstimulus intervals (Santistevan et al., 2022; Wolman et al., 2011). For the sensitive step, a moderate level acoustic stimulus (1000 Hz, 100 μs and 91.9 dB re 20 μPa), able to elicit ASR in about 30 % of the population, was selected, while a strong startle-inducing stimulus (1000 Hz, 1 ms, 103.9 dB re 20 μPa) able to elicit ASR in about 70 % of the animals was selected for the prehabituation, habituation and recovery steps for the habituation protocol in adult zebrafish (Fig. 4A). Series of five stimuli were delivered during the sensitivity, prehabituation, and recovery steps, with interstimulus intervals (ISI) of 120 s. For the habituation step, a series of 15 stimuli was delivered every 2 s. Fig. 4B summarizes results from habituation experiments in a total of 10 groups of adult zebrafish (51 fish in total; results of each experiment can be found in Supplementary Dataset 2). The proposed protocol provides baselines for the sensitivity to the acoustic stimuli (first step), the highest ASR percentage of the batch (second step), the short-term habituation to a series of startle-inducing stimuli (third step) and, after 5 min of resting time, the recovery of the responses of the adult fish to a series of startle-inducing stimuli (fourth step).

The results of habituation experiments are commonly presented as percentage of responses to the different steps and percentage of habituation. To determine the percentage of habituation in larvae, Wolman et al. (2011) proposed to calculate the ratio between ASR during the last 10 stimuli of the habituation step and the 10 stimuli delivered in the prehabituation step. The percentage of ASR habituation in adult zebrafish was initially calculated as the ratio of the responses during the first (stimuli 1–5), second (stimuli 6–10), or last (stimuli 11–15) 5-stimulus periods of the habituation step to the responses during the 5 stimuli delivered during the prehabituation step (Fig. 4C and Supplementary Dataset 3). The results show a 62.5 % habituation (IQR: 28.6–84.8 %) during the initial 5 stimuli of the series, and then, habituation increases up to 85–88 % during the 6–10 or 11–15 stimuli of the series (Fig. 4C). Zebrafish larvae also reach the habituation by the 11th–15th stimulus



C

	Latency (ms)	Max Angular Vel. (°/ms)	C-bend duration (ms)	C-bend angle (°)
ASR median (IQR)	10 (9-11)	18.4 (16.5-20.8)	10 (9-12)	95.8 (84.4-105.6)

Fig. 3. Kinematic values of 1875 acoustic startle responses (ASR) in adult zebrafish. Histograms of latency (A) and C-bend angle (B), and the main kinematic values of these responses (C). IQR: interquartile range.

during the habituation period (Wolman et al., 2011). Therefore, when similar criteria were used for determining the short-term ASR habituation in adult and larval zebrafish, similar levels of habituation are reached (Jain et al., 2018; Wolman et al., 2011, 2015).

3.3. Prepulse inhibition of ASR in adult zebrafish

As other vertebrate models, zebrafish larvae can modulate ASR by sensorimotor gating, and both custom-made (Bhandiwad et al., 2013, 2018; Burgess and Granato, 2007a; Scaramella et al., 2022) and commercial (Banono and Esguerra, 2020) systems have been developed for quantitatively determining this neuromodulatory process through the PPI analysis. However, to the best of our knowledge, there are currently no platforms available to assess PPI in adult zebrafish and, as a result, information on sensorimotor gating in adults remains quite limited (Burgess and Granato, 2007a). Therefore, the suitability of Zebra_K to determine PPI in adult zebrafish was tested (Fig. 5A). First of all, an AV stimulus of 1000 Hz, 10 μs and 72.9 dB re 20 μPa, which typically elicited 0–10 % startle responses, was selected as a prepulse. The startle-inducing stimulus used in the prehabituation step of the habituation protocol (1000 Hz, 1 ms, 103.9 dB re 20 μPa) was also selected for the pulse. The developed PPI protocol started with 5 prepulse stimuli (ISI: 120 s), followed by 5 pulse (ISI: 120 s) and finally by a series of 5 sequences prepulse+pulse ($ISI_{between\ sequences}$: 120 s), with the interval between prepulse and pulse to be determined.

Fig. 5A shows that the median ASR frequencies during the prepulse and pulse steps were 0 % (IQR: 0–0 %) and 72.2 % (IQR: 61.1–77.8 %), respectively (individual data provided in Supplementary Dataset 4). A significant inhibition of the startle response, with only a 22.2 % of responses (IQR: 8.3–36.1 %), was found when the prepulse was delivered 100 ms before the pulse ($U(N_{pulse} = 10, N_{prepulse+pulse} = 10) = 4.500$, $z = -3.471$, $P = 1.3 \times 10^{-4}$), with a median PPI percentage of 67.2 % (IQR: 46.7—87.7 %). This PPI percentage is consistent with the reported by Burgess and Granato (2007a) for wild-type adult fish.

The effect of the interval between prepulse and pulse on the PPI magnitude was then analyzed. Whereas the pulse led a median ASR frequency of 77.8 % (IQR: 66.7–88.9 %) of the fish, this frequency decreased to 25.0 % (IQR: 11.1–40 %) or 44.4 % (IQR: 25.0–55.6 %) if a prepulse was delivered 500 or 1000 ms before the pulse, respectively. In contrast, when the prepulse was delivered 1 ms or 2000 ms before the pulse, it failed to decrease the ASR frequency. As shown in Fig. 5B, PPI % was maximal at intervals between 10 and 100 ms (data provided as Supplementary Dataset 5). The interval range leading to a significant PPI in adult zebrafish in this study, and the interval resulting in the maximal PPI% (50 ms), are consistent with the results reported elsewhere with wild-type adult zebrafish (Burgess and Granato, 2007a). However, in contrast to our results, no PPI was found in Tuebingen long-fin adult zebrafish when the prepulse was delivered 10 or 1000 ms before the pulse (Burgess and Granato, 2007a). Differences in the genetic background and/or the type of stimuli used could explain the wider range of intervals leading PPI in our study.

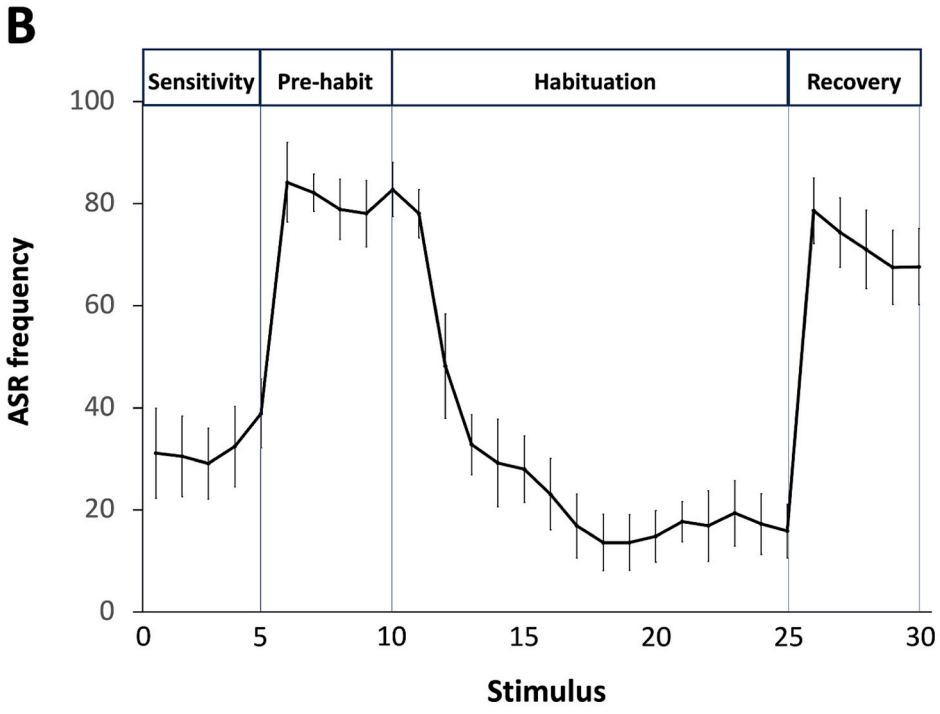
3.4. Physiological modulation of ASR sensitivity and plasticity by the time of day in adult zebrafish

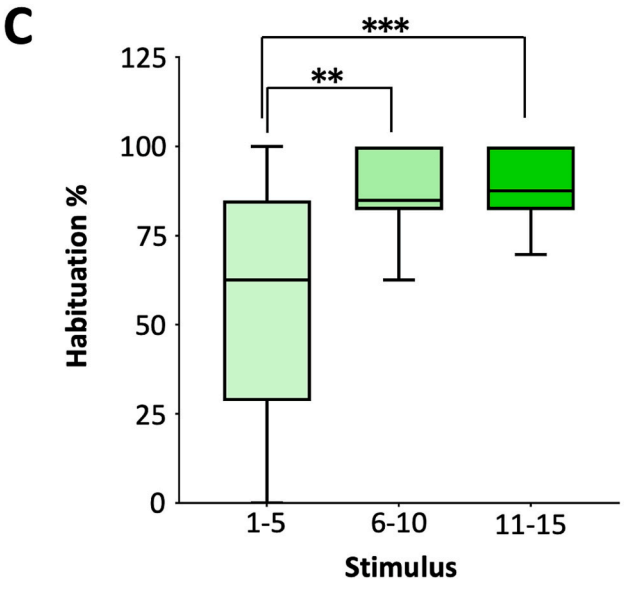
To ensure the reliability and replicability of the developed assays, it is important to identify the intrinsic factors that influence ASR sensitivity and plasticity in adult zebrafish. Among the intrinsic factors, the time of day is known to strongly influence the outcome of many behavioral assays in zebrafish (Tagkalidou et al., 2024). Whereas de effects of the time of day on ASR and its plasticity has been studied in rodents (Chabot and Taylor, 1992; Horlington, 1970; Kinkead et al., 2008) and human (Miller and Gronfier, 2006), the potential effect of this intrinsic factor in zebrafish has not been analyzed yet. Therefore, we have analyzed the effect of the time of day on the results of the habituation and PPI protocols in adult zebrafish.

As Fig. 6A-B shows, when the effect of the time of day on the habituation protocol was analyzed between 10:00 am and 06:00 pm, no

A

	Sensitivity	Prehabituation	Habituation		Recovery
Stimulus	1000 Hz, 100 μs, 91.9 dB	100 Hz, 1 ms, 103.9 dB	100 Hz, 1 ms, 103.9 dB	Resting (300 sec)	1000 Hz, 1 ms, 103.9 dB
Number of stimuli	5	5	15		5
ISI (s)	120	120	2		120





(caption on next page)

Fig. 4. Adult zebrafish habituation to acoustic stimuli using Zebra_K. (A) Main steps of the habituation of acoustic startle response (ASR) protocol optimized for adult zebrafish. (B) Trace of the mean ASR frequencies in adult zebrafish using the developed protocol. Results of 10 groups of fish (51 fish in total), presented as mean ± SEM. (C) ASR habituation calculated as the ratio of the responses during the first (1–5), second (6–10), or last (11–15) stimuli during the habituation step to the responses during the 5 stimuli delivered during the prehabituation step. Data are presented as boxplots where the box indicates the 25th and 75th percentiles, the thin line within the box marks the median and the whiskers the maximum and minimum values. $^{**}P < 0.01$, $^{***}P < 0.001$; Kruskal Wallis test with Bonferroni correction. Data from 3 independent experiments.

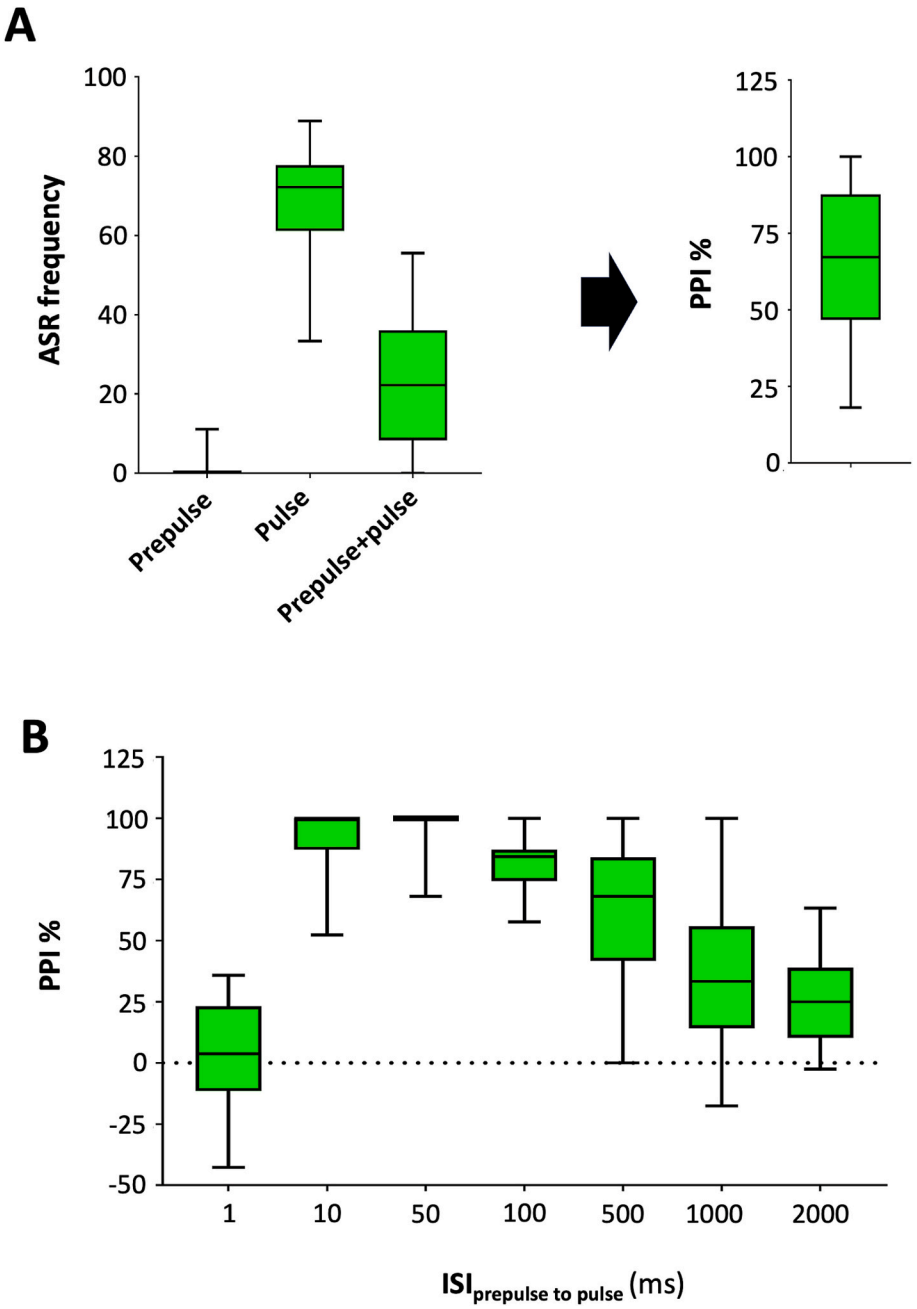


Fig. 5. Prepulse inhibition of the acoustic startle response (ASR) in adult zebrafish. (A) ASR frequency during the prepulse, pulse and the prepulse+pulse sequence. In each experiment 9 adult zebrafish were acclimated to the Zebra_K observation chamber and then, 5 prepulses of mild acoustic stimuli were delivered with an interstimulus interval (ISI) of 120 s. Then, 5 strong acoustic stimuli pulses followed by 5 prepulse+pulse sequences were delivered using the same ISI. In each sequence, the interval between the prepulse and the pulse was 100 ms. The boxplot on the right represents the percentage of PPI, calculated from the ASR during the pulse and during the prepulse+pulse sequences. (B) Analysis of the effect of different interstimulus intervals (ISI) between prepulse and pulse on the percentage of PPI. Data are presented as boxplots where the box indicates the 25th and 75th percentiles, the thin line within the box marks the median and the whiskers the maximum and minimum values. Data from 2 to 4 independent experiments with 9 adult wild-type short-fin zebrafish each.

significant effects were found for the sensitivity step. However, ASR frequency during the prehabituation step was significantly lower at 06:00 pm [80.0 % (IQR: 60.0–83.3 %)] than at 02:00 pm [85.7 % (IQR: 83.3–100.0 %) and 04:00 pm [92.9 % (IQR: 85.7–100.0 %)] [$H(3, N = 55) = 15.427$, $P = 0.0015$; 02:00 pm vs 06:00 pm: $P = 0.017$, and 04:00 pm vs 06:00 pm: $P = 0.003$]. Moreover, ASR frequency during the last

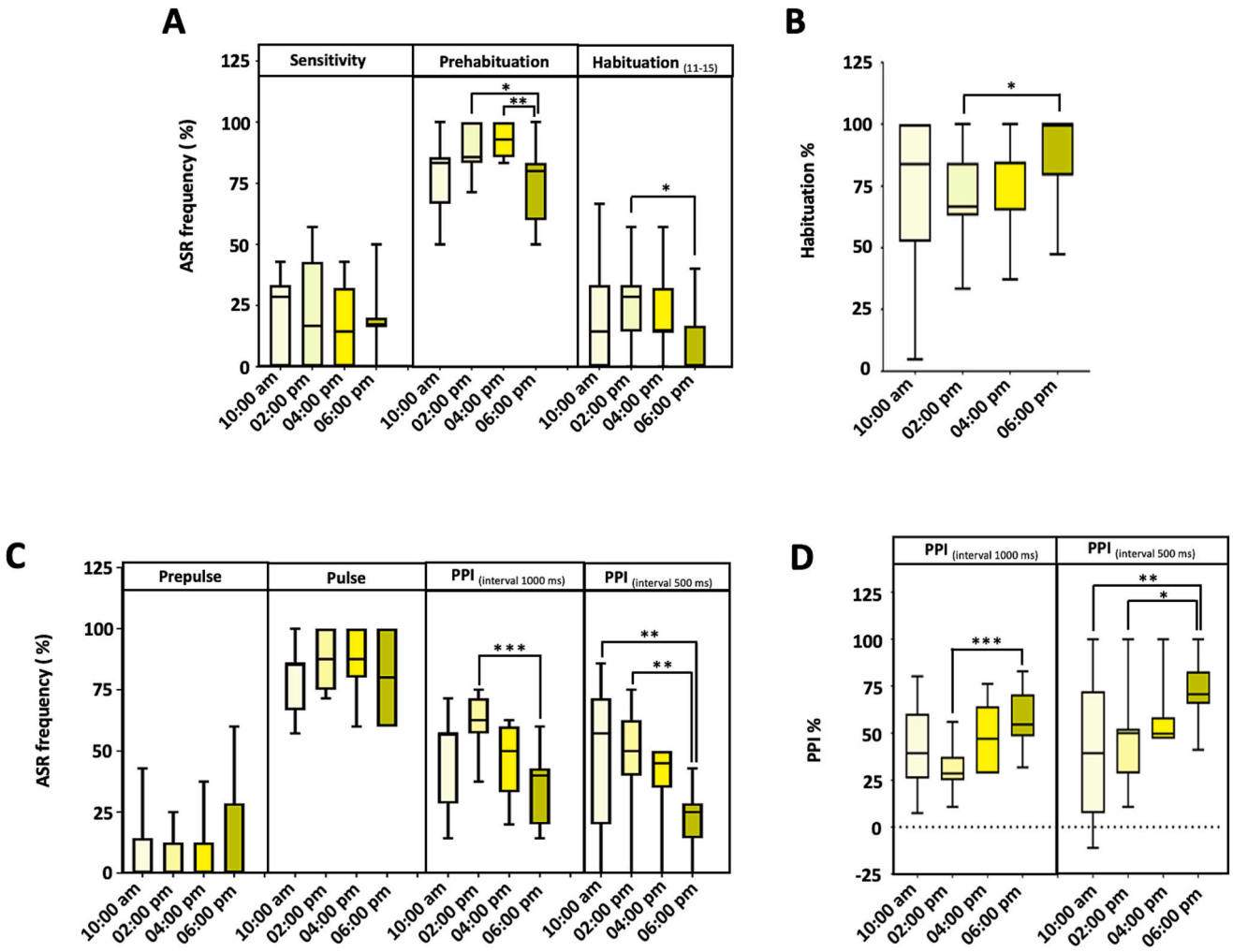


Fig. 6. Effect of the time of day on acoustic startle response (ASR) plasticity in adult zebrafish. (A) The analysis of the effect of time of day on ASR frequency during the habituation protocol shows a significant decrease when the analysis was performed at 06:00 pm compared to that at 02:00 pm (prehabituation and habituation steps) and 04:00 pm (prehabituation step); (B) The percentage of habituation is significantly higher when the analysis was performed at 06:00 pm compared to that at 02:00 pm; (C) The analysis of the effect of time of day on ASR frequency during the prepulse inhibition (PPI) protocol shows a significant decrease in ASR frequency when the analysis was performed at 06:00 pm compared to that at 02:00 pm (interval prepulse-to-pulse: 1000 and 500 ms) and 10:00 am (intervals prepulse-to-pulse: 500 ms); (D) The PPI percentage was significantly higher when the analysis was performed at 06:00 pm compared to that at 02:00 pm (interval prepulse-to-pulse: 1000 and 500 ms) and 10:00 am (intervals prepulse-to-pulse: 500 ms). Data are presented as boxplots where the box indicates the 25th and 75th percentiles, the thin line within the box marks the median and the whiskers the maximum and minimum values. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; Kruskal Wallis test with Bonferroni correction. Data from 2 to 3 independent experiments.

period of habituation (stimuli 11–15) at 06:00 pm [0 % (IQR: 0.0–16.7 %)] was significantly lower than the corresponding at 02:00 pm [28.6 % (IQR: 14.3–33.3 %)] [$H(3, N = 55) = 9.319$, $P = 0.025$; 02:00 pm vs 06:00 pm: $P = 0.016$]. Finally, habituation percentage measured at 06:00 pm [100 % (IQR: 79.17–100.0 %)] was significantly higher than it was measured at 02:00 pm [66.7 % (IQR: 63.0–84.4 %)] [$H(3, N = 55) = 8.358$, $P = 0.039$; 02:00 pm vs 06:00 pm: $P = 0.028$]. These results show a significant effect of time of day on the results of habituation experiments, which is restricted to experiments performed at 06:00 pm. The period from 10:00 to 16:00 is suitable for habituation experiments, since no differences in sensitivity, prehabituation and habituation results were found within this period.

The effect of time of day on the results of the PPI experiments was also analyzed. As shown in Fig. 6C-D, the time of day had no significant effect on the ASR frequency during prepulse or pulse. However, the ASR frequency during PPI was lower [$H(3, N = 55) = 16.459$, $P = 0.0009$; 02:00 pm vs 06:00 pm: $P = 0.0003$] and the PPI percentage was higher [$H(3, N = 55) = 15.726$, $P = 0.0013$; 02:00 pm vs 06:00 pm: $P = 0.0009$] at 06:00 pm than at 02:00 pm using 1000 ms as prepulse-to-pulse intervals. A similar effect of the time of day on ASR frequency [$H(3, N = 55) = 14.909$, $P = 0.0019$; 02:00 pm vs 06:00 pm: $P = 0.007$, and 10:00 am vs 06:00 pm: $P = 0.004$] and PPI percentage [$H(3, N = 55) = 13.100$, $P = 0.004$; 02:00 pm vs 06:00 pm: $P = 0.023$, and 10:00 am vs 06:00 pm: $P = 0.006$] was found when 500 ms was used as prepulse-to-pulse interval. Therefore, these results show a significant effect of time of day on the results of the PPI experiments and, similar to what was described above for habituation, this effect is limited to the experiments conducted at 06:00 pm. The period between 10:00 am and 16:00 pm is also suitable for PPI experiments with Zebra_K.

Based on these results demonstrating the effect of time on ASR results and its plasticity in adult zebrafish, all the rest of the experiments presented in this work were performed in the period between 10:00 am and 04:00 pm.

3.5. Physiological modulation of ASR sensitivity and plasticity by gender in adult zebrafish

While a significative effect of gender on ASR modulation has been

reported in humans (Kofler et al., 2001), no information is currently available for zebrafish. The detailed results of the modulation of ASR sensitivity and plasticity by fish gender are provided in Supplementary Dataset 6. Fig. 7A shows that females exhibit a higher responsiveness than males [$U(N_{females} = 30, N_{males} = 30) = 640.000$, $z = 2.954$, $P = 0.003$] to acoustic stimuli from mild to moderate level [50.0 % (25.0–75.0 %) vs 25.0 % (25.0–31.2 %) for females and males, respectively] during the sensitivity step of the habituation protocol. Similarly, females exhibit a higher responsiveness [$U(N_{females} = 30, N_{males} = 30) = 771.000$, $z = 5.013$, $P = 5.4 \times 10^{-7}$] to the strong acoustic stimuli used for the prehabituation step [100.0 % (75.0–100.0 %) vs 50.0 % (50.0–75.0 %) for females and males, respectively]. Moreover, as shown in Fig. 7B, females exhibit a significatively lower habituation than males to a series of acoustic stimuli [70.6 % (41.2–73.7 %) vs 100.0 % (61.5–100.0 %) habituation percentage for females and males, respectively; $U(N_{females} = 30, N_{males} = 30) = 201.000$, $z = -3.764$, $P = 1.7 \times 10^{-4}$). These results are consistent with the reported gender effect on ASR in humans, where women exhibit higher responsiveness to ASR and lower habituation than men (Kofler et al., 2001).

The effect of gender on sensorimotor gating of ASR was studied using the PPI protocol. As shown in Fig. 7D (Supplementary Dataset 6), females exhibit increased responsiveness to the acoustic stimulus used for the pulse [100.0 % (75.0–100.0 %) vs 50.0 % (43.8–81.2 %) of ASR for females and males, respectively; $U(N_{females} = 30, N_{males} = 30) = 727.000$, $z = 4.389$, $P = 1.1 \times 10^{-5}$]. This result is consistent with what was observed during the prehabituation step, since in both cases the same stimulus was used. Finally, Fig. 7D shows that PPI percentage is significantly lower in females than in males [45.6 % (24.5–67.6 %) vs 100 % (59.7–100.0 %) of PPI % in females and males, respectively; $U(N_{females} = 30, N_{males} = 30) = 198.000$, $z = -3.789$, $P = 1.5 \times 10^{-4}$], consistent with the lower values of PPI reported in females both in humans (Swerdlow et al., 1993) and rodent models (Lehmann et al., 1999) compared to males.

3.6. Pharmacological modulation of ASR sensitivity and plasticity by the NMDA receptor antagonist ketamine in adult zebrafish

In both mammalian models and zebrafish larvae, behavioral plasticity of ASR is modulated by *N*-methyl-D-aspartate (NMDA) receptor antagonists (Burgess and Granato, 2007a; Koch, 1999; Wolman et al., 2011). However, no information is currently available about the modulation of ASR by NMDA-receptor antagonists in adult zebrafish. To evaluate the specific effects of NMDA receptor antagonists on habituation and PPI, a pretest-posttest control group design [25] with ketamine was employed.

The detailed results of the modulation of ASR sensitivity and plasticity by ketamine are provided in the Supplementary Dataset 7. As shown in Fig. 8A, no differences in sensitivity were found between the control [20.0 % (IQR: 0.0–40.0 %)] and ketamine [20.0 % (IQR: 0.0–25.0 %)] groups before starting the treatment (pretest). Then, sensitivity was determined again in these groups 20 min after waterborne exposure to fish water (control group) or 50 μM of the NMDA-receptor antagonist ketamine (ketamine group; posttest). While no significant changes were found in the control group [25 % (IQR:25–40 %), $U(N_{before} = 15, N_{after} = 14) = 122.00$, $z = 0.765$, $P = 0.477$]], a significant increase in the responsiveness was found during the sensitivity step of the habituation protocol in the ketamine-treated group [50 % (IQR:40–75 %), $U(N_{before} = 15, N_{after} = 14) = 197.00$, $z = 4.063$, $P = 9.6 \times 10^{-6}$]. Wolman et al. (2011) reported a similar increase in responsiveness to ASR in zebrafish larvae after the exposure to the NMDA receptor antagonists MK-801 and ketamine. Interestingly, this effect of the ketamine in larvae was observed at a ketamine concentration 10-fold higher than that used with adults in our study. In addition to increasing sensitivity to the acoustic stimulus, ketamine exposure also led a strong decrease in the habituation percentage [$U(N_{before} = 15, N_{after} = 15) = 1.000$, z = −4.700, $P = 2.6 \times 10^{-8}$)]. As shown in Fig. 8B,

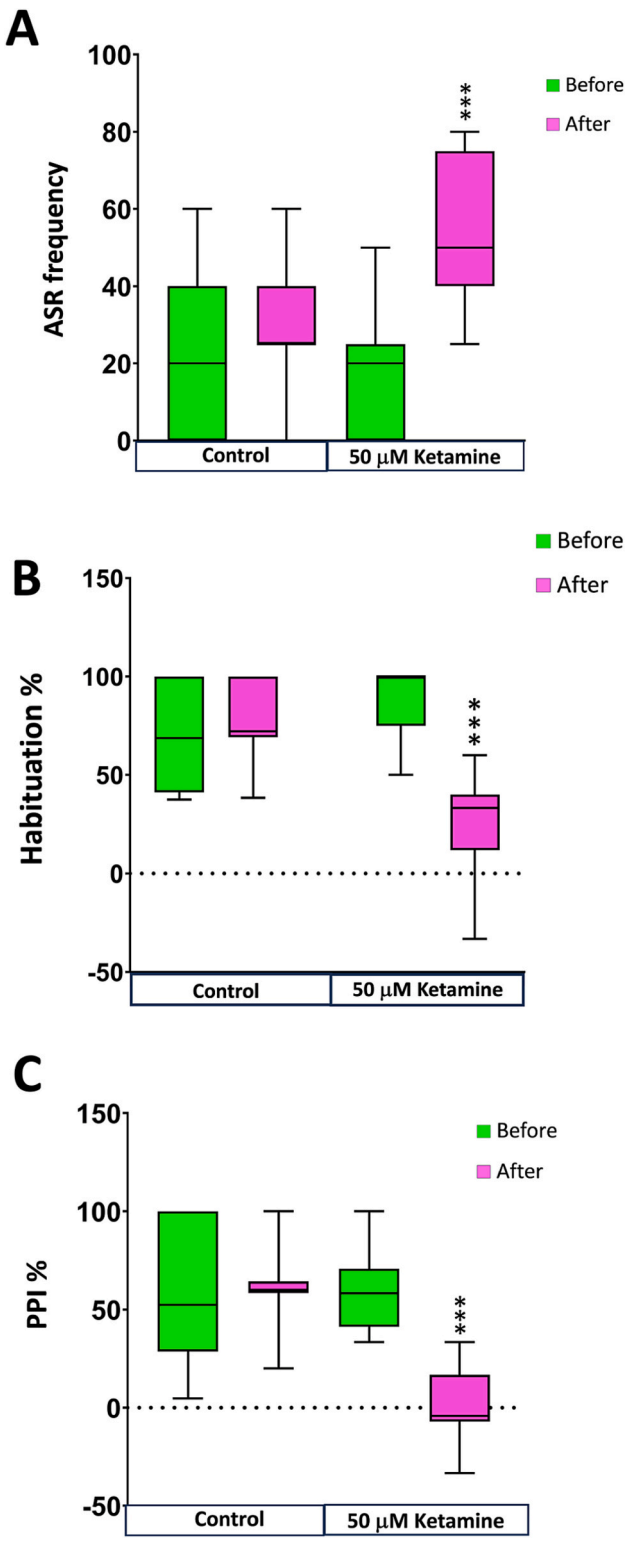


Fig. 7. Effect of 20 min exposure to 50 μM ketamine on acoustic startle response (ASR) plasticity in adult zebrafish using a pretest-posttest control group design. Exposure to ketamine increases responsiveness to moderate-level stimuli during the sensitivity step of the habituation protocol (A), decreases the percentage of habituation to a series of strong-level acoustic stimuli (B) and leads to the total abolition of the prepulse inhibition (C). Data are presented as boxplots where the box indicates the 25th and 75th percentiles, the thin line within the box marks the median and the whiskers the maximum and minimum values. Mann–Whitney *U* test, *** $P < 0.001$. Data from 3 independent experiments with 4–5 adult wild-type short-fin zebrafish by experimental group in each experiment.

while no differences were found in the control groups before and after treatment [$U(N_{before} = 15, N_{after} = 15) = 151.000$, z = 1.641, $P = 0.116$)], the percentage of habituation decreased from 100 % (75–100 %) to 33.3 % (11.8–40 %) after ketamine treatment. These results on the effect of this NMDA receptor antagonist on the short-term habituation of ASR in adult zebrafish are consistent with the reported decrease in habituation evoked by ketamine and MK-801 in zebrafish larvae (Wolman et al., 2011).

In order to determine the effect of ketamine on the PPI percentage of adult zebrafish, a 500 ms interstimulus interval between the prepulse and the pulse was used. As shown in Fig. 8C, while no differences were observe in the control groups before and after treatment [$U(N_{before} = 15, N_{after} = 15) = 151.500$, z = 1.632, $P = 0.106$)], a total abolition of the PPI was found 20 min after exposure to 50 μM ketamine [$U(N_{before} = 15, N_{after} = 15) = 0.500$, z = −4.667, $P = 1.3 \times 10^{-8}$)]. These results are consistent with an strong decrease in PPI% previously reported in zebrafish larvae exposed to 100 μM ketamine for 10 min using the same prepulse-to-pulse interval (Burgess and Granato, 2007a). However, the effect of ketamine on PPI appears to depend on the prepulse-to-pulse interval used, with intervals of 30 or 100 ms reported to increase the PPI percentage in ketamine exposed larvae (Banono and Esguerra, 2020; Burgess and Granato, 2007a). The observed effect of ketamine on sensorimotor gating in adult zebrafish in this study is also consistent with the reported effect of this NMDA receptor antagonist on PPI in different mammalian models (Canal et al., 2001; Mansbach and Geyer, 1991; Sandner et al., 2002).

3.7. Reliability and validity of Zebra_K automated platform

First of all, the accuracy of the Zebrafish Acoustic Startle Response kinematic analysis software in determining kinematic parameters was determined by analyzing fifty videos of ACR using both manual

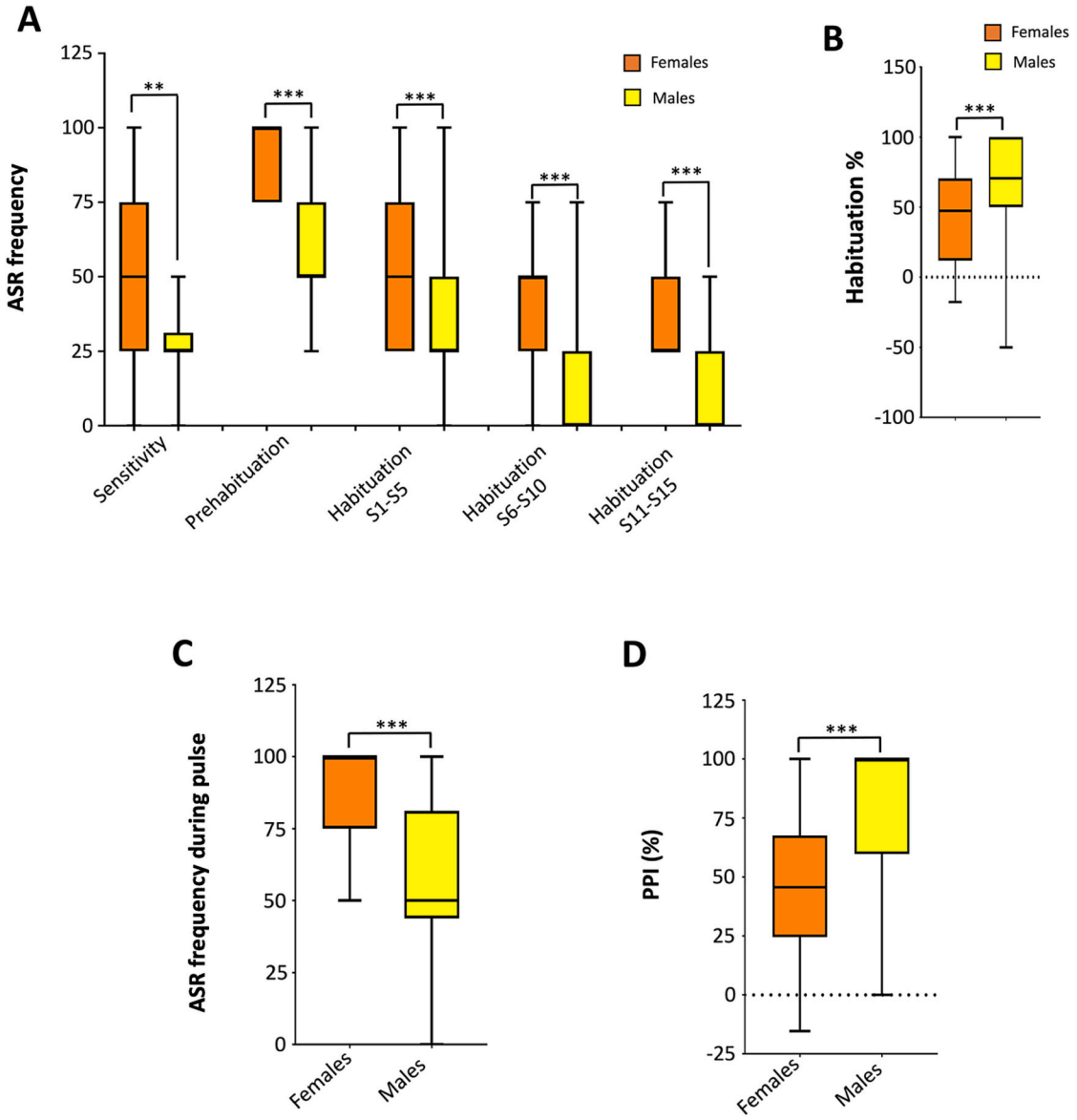


Fig. 8. Effect of gender on ASR plasticity in adult zebrafish. Female fish exhibited higher responsiveness than males to the acoustic stimuli through the habituation protocol (A), and a decreased habituation to a series of strong level acoustic stimuli compared to males (B). Consistent to the higher responsiveness in the habituation protocol, females also exhibited a higher responsiveness to the pulse than males (C). Finally, prepulse inhibition in females was significantly lower than in males (D). Data are presented as boxplots where the box indicates the 25th and 75th percentiles, the thin line within the box marks the median and the whiskers the maximum and minimum values. $^{**}P < 0.01$, $^{***}P < 0.001$; Mann–Whitney U test. Data from 3 independent experiments with 4–5 adult wild-type short-fin zebrafish by experimental group in each experiment.

assessment and the automated tracking software. As shown in Supplementary Table S2, the results from the manual and automated methods were very similar, confirming that the automated tracking software provides reliable measurements of kinematic parameters. Similar correlation between manual and automated (FLOTE) kinematic measures of ASR was obtained by Burgess and Granato (2007a, 2007b) with zebrafish larvae. Despite the strong correlation observed between manual analysis and software analysis, there may be specific instances where the software introduces errors. However, the software interface has been designed to facilitate the identification of potential errors in the reaction graphs and provides all the necessary information for the manual correction of any detected inaccuracies.

Statistical results of comparing the performance of up to 20 different groups of fishes, 15 of them with different fishes across up to 8 different times (days) are shown in Supplementary Table S3 and Supplementary Figs. S3-S4. For sensitivity, prehabituation and the second and third periods of habituation, significant differences were found among groups or fishes but not across days. The exception was the observed significant effect of day for PPI (ISI: 0.5 s) in the first model. The variability accounted for group or fish, which can be considered an intra-day one, was similar and in most cases (12 of 16) below 30 %. Inter-day variability was in below 10 % in 14 of 16 occasions.

Construct validity of the Zebra_K platform has been demonstrated throughout this manuscript by showing that adult zebrafish display ASR, habituation, and PPI in ways consistent with previous findings in zebrafish larvae, rodents, and other model organisms (Banono and Esguerra, 2020; Burgess and Granato, 2007a; Gómez-Nieto et al., 2020; Wolman et al., 2011). The kinematic parameters of ASR in adults closely resemble those reported for SLC in larvae (Burgess and Granato, 2007b), and short-term habituation and PPI patterns observed in adult zebrafish align with these modulatory processes in larvae and other vertebrate models (Burgess and Granato, 2007a; Canal et al., 2001; Gómez-Nieto et al., 2020; Mansbach and Geyer, 1991; Sandner et al., 2002; Wolman et al., 2011). These behaviors are well-established constructs in behavioral neuroscience, supporting that our platform accurately measures ASR and sensorimotor gating. Furthermore, pharmacological manipulation with the NMDA antagonist modulates habituation and PPI as expected, reinforcing that our platform's measures align with established neurobiological responses (Burgess and Granato, 2007a; Wolman et al., 2011).

Moreover, convergent validity of the platform is supported by its ability to generate similar kinematic parameters (e.g., latency, bending, angular velocity) to those observed for SLC in zebrafish larvae using automated kinematic analysis platforms (Burgess and Granato, 2007a, 2007b). This consistency indicates that results provided by Zebra_K align with established findings in similar paradigms. Additionally, the effect of gender observed in adult zebrafish mirrors findings in rodent models (Lehmann et al., 1999; Swerdlow et al., 1993), indicating the platform's sensitivity to biologically meaningful factors.

Finally, predictive validity of Zebra_K is demonstrated by the platform's ability to produce expected results in response to ketamine, an NMDA antagonist known to modulate sensorimotor gating. This predictable modulation aligns with theoretical expectations, confirming that Zebra_K can reliably predict behavioral outcomes based on established neurobiological models.

4. Conclusions

The present study shows a kinematic analysis automated platform presented for assessing ASR and its plasticity in short-fin wild type adult zebrafish. Protocols have been developed and validated for determining sensitivity, habituation and PPI of the ASR, finding that the most stable period of the day to perform these experiments is between 10:00 am and 04:00 pm. When the effect of the gender was tested, females exhibited higher responsiveness to the startle and lower habituation% and sensorimotor gating than males. Finally, ASR plasticity was modulated by the NMDA receptor agonist ketamine in a similar fashion than in other vertebrate models. These results emphasize the interest of using adult zebrafish to assess the potential effect of environmentally relevant neuroactive/neurotoxic chemicals on the ASR and its plasticity, especially in studies that cannot be performed in embryos, such as studying the long-term effects of developmental exposure to neuroactive/neurotoxic compounds or the gender-specific effect of some environmental pollutants.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.178028.

CRediT authorship contribution statement

Marija Stevanović: Writing – review & editing, Writing – original draft, Visualization, Investigation. **Niki Tagkalidou:** Writing – review & editing, Writing – original draft, Investigation. **Cristiana Roberta Multisanti:** Writing – review & editing, Writing – original draft, Investigation. **Sergi Pujol:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Conceptualization. **Ouwais Aljabasini:** Writing – review & editing, Writing – original draft, Investigation. **Eva Prats:** Writing – review & editing, Writing – original draft, Investigation. **Caterina Faggio:** Writing – review & editing, Writing – original draft, Supervision, Resources, Funding acquisition. **Josep Maria Porta:** Writing – review & editing, Writing – original draft, Supervision, Software, Methodology, Conceptualization. **Carlos Barata:** Formal analysis, Writing – review & editing. **Demetrio Raldúa:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Informed consent statement

Not applicable.

Institutional review board statement

All procedures were approved by the Institutional Animal Care and Use Committees at the CID-CSIC (OH 1032/2020) and conducted in accordance with the institutional guidelines under a license from the local government (agreement number 11336).

Funding

This work was carried out in the framework of the European Partnership for the Assessment of Risks from Chemicals (PARC) and has received funding from the European Union's Horizon Europe research and innovation programme under Grant Agreement No 101057014, by "Agencia Estatal de Investigación" from the the Ministry of Science, Innovation and Universities, (PID2023-148502OB-C21 and PDC2021-120754-I00), by the NATO Science for Peace and Security Program—SPS.MYP G5852, and by IDAEA-CSIC, Severo Ochoa Centre of Excellence (CEX2018-000794-S).

Declaration of competing interest

The authors declare no conflicts of interest.

Data availability statement

The data supporting the findings of this study are available within the manuscript and its Supplementary Material file or will be made available from the corresponding author upon request.

References

Babin, P.P.J., Goizet, C., Raldúa, D., 2014. Zebrafish models of human motor neuron diseases: advantages and limitations. Prog. Neurobiol. 118, 36–58.

Banono, N.S., Esguerra, C.V., 2020. Pharmacological validation of the prepulse inhibition of startle response in larval zebrafish using a commercial automated system and software. J. Vis. Exp. 2020, 1–18. https://doi.org/10.3791/61423.

Bhandiwad, A.A., Zeddies, D.G., Raible, D.W., Rubel, E.W., Sisneros, J.A., 2013. Auditory sensitivity of larval zebrafish (Danio rerio) measured using a behavioral prepulse inhibition assay. J. Exp. Biol. 216, 3504–3513. https://doi.org/10.1242/jeb.087635.

Bhandiwad, A.A., Raible, D.W., Rubel, E.W., Sisneros, J.A., 2018. Noise-induced hypersensitization of the acoustic startle response in larval zebrafish. JARO - J. Assoc. Res. Otolaryngol. 19, 741–752. https://doi.org/10.1007/s10162-018-00685-0.

Brun, N.R., Panlilio, J.M., Zhang, K., Zhao, Y., Ivashkin, E., Stegeman, J.J., Goldstone, J. V., 2021. Developmental exposure to non-dioxin-like polychlorinated biphenyls promotes sensory deficits and disrupts dopaminergic and GABAergic signaling in zebrafish. Commun. Biol. 4. https://doi.org/10.1038/s42003-021-02626-9.

Burgess, H.A., Granato, M., 2007a. Sensorimotor gating in larval zebrafish. J. Neurosci. 27, 4984–4994. https://doi.org/10.1523/JNEUROSCI.0615-07.2007.

Burgess, H.A., Granato, M., 2007b. Modulation of locomotor activity in larval zebrafish during light adaptation. J. Exp. Biol. 210, 2526–2539. https://doi.org/10.1242/jeb.003939.

Canal, N.M., Gourevitch, R., Sandner, G., 2001. Non-monotonic dependency of PPI on temporal parameters: differential alteration by ketamine and MK-801 as opposed to apomorphine and DOI. Psychopharmacology 156, 169–176. https://doi.org/10.1007/s002130100809.

Chabot, C.C., Taylor, D.H., 1992. Daily rhythmicity of the rat acoustic startle response. Physiol. Behav. 51, 885–889. https://doi.org/10.1016/0031-9384(92)90131-K.

Chen, L., Toth, M., 2001. Fragile X mice develop sensory hyperreactivity to auditory stimuli. Neuroscience 103, 1043–1050. https://doi.org/10.1016/S0306-4522(01)00036-7.

Dimitrov, D.M., Rumrill, P.D., 2003. Pretest-posttest designs and measurement of change. Work 20, 159–165.

Fero, K., Yokogawa, T., Burgess, H.A., 2011. The behavioral repertoire of larval zebrafish. Zebrafish Models in Neurobehavioral Research. Springer 249–291.

Gómez-Nieto, R., de Horta-Júnior, J. de A.C., Castellano, O., Millian-Morell, L., Rubio, M. E., López, D.E., 2014. Origin and function of short-latency inputs to the neural substrates underlying the acoustic startle reflex. Front. Neurosci. 8, 1–18. https://doi.org/10.3389/fnins.2014.00216.

Gómez-Nieto, R., Hormigo, S., López, D.E., 2020. Prepulse inhibition of the auditory startle reflex assessment as a hallmark of brainstem sensorimotor gating mechanisms. Brain Sci. 10, 1–15. https://doi.org/10.3390/brainsci10090639.

Hoenig, K., Hochrein, A., Quednow, B.B., Maier, W., Wagner, M., 2005. Impaired prepulse inhibition of acoustic startle in obsessive-compulsive disorder. Biol. Psychiatry 57, 1153–1158. https://doi.org/10.1016/j.biopsych.2005.01.040.

Horlington, M., 1970. Startle response circadian rhythm in rats: lack of correlation with motor activity. Physiol. Behav. 5, 49–53. https://doi.org/10.1016/0031-9384(70)90012-0.

Jafari, Z., Kolb, B.E., Mohajerani, M.H., 2020. Prepulse inhibition of the acoustic startle reflex and P50 gating in aging and alzheimer's disease. Ageing Res. Rev. 59, 101028. https://doi.org/10.1016/j.arr.2020.101028.

Jain, R.A., Wolman, M.A., Marsden, K.C., Nelson, J.C., Shoenhard, H., Echeverry, F.A., Szi, C., Bell, H., Skinner, J., Cobbs, E.N., Sawada, K., Zamora, A.D., Pereda, A.E., Granato, M., 2018. A forward genetic screen in zebrafish identifies the G-protein-coupled receptor CaSR as a modulator of sensorimotor decision making. Curr. Biol. 28, 1357–1369.e5. https://doi.org/10.1016/j.cub.2018.03.025.

Kinkead, B., Yan, F., Owens, M.J., Nemeroff, C.B., 2008. Endogenous neurotensin is involved in estrous cycle related alterations in prepulse inhibition of the acoustic startle reflex in female rats. Psychoneuroendocrinology 33, 178–187. https://doi.org/10.1016/j.psyneuen.2007.11.005.

Koch, M., 1999. The neurobiology of startle. Prog. Neurobiol. 59, 107–128.

Kofler, M., Müller, J., Reggiani, L., Valls-Solé, J., 2001. Influence of gender on auditory startle responses. Brain Res. 921, 206–210. https://doi.org/10.1016/S0006-8993(01)03120-1.

Lauer, A.M., Behrens, D., Klump, G., 2017. Acoustic startle modification as a tool for evaluating auditory function of the mouse: Progress, pitfalls, and potential. Neurosci. Biobehav. Rev. 77, 194–208. https://doi.org/10.1016/j.neubiorev.2017.03.009.

Lauer, J., Zhou, M., Ye, S., Menegas, W., Schneider, S., Nath, T., Rahman, M.M., Di Santo, V., Soberanes, D., Feng, G., Murthy, V.N., Lauder, G., Dulac, C., Mathis, M.W., Mathis, A., 2022. Multi-animal pose estimation, identification and tracking with DeepLabCut. Nat. Methods 19, 496–504. https://doi.org/10.1038/s41592-022-01443-0.

Lehmann, J., Pryce, C.R., Feldon, J., 1999. Sex differences in the acoustic startle response and prepulse inhibition in Wistar rats. Behav. Brain Res. 104, 113–117. https://doi.org/10.1016/S0166-4328(99)00058-3.

Leng, A., Yee, B.K., Feldon, J., Ferger, B., 2004. Acoustic startle response, prepulse inhibition, and spontaneous locomotor activity in MPTP-treated mice. Behav. Brain Res. 154, 449–456. https://doi.org/10.1016/j.bbr.2004.03.012.

López-Schier, H., 2019. Neuroplasticity in the acoustic startle reflex in larval zebrafish. Curr. Opin. Neurobiol. 54, 134–139. https://doi.org/10.1016/j.conb.2018.10.004.

Mansbach, R.S., Geyer, M.A., 1991. Parametric determinants in pre-stimulus modification of acoustic startle: interaction with ketamine. Psychopharmacology 105, 162–168. https://doi.org/10.1007/BF02244303.

Mathis, A., Mamidanna, P., Cury, K.M., Abe, T., Murthy, V.N., Mathis, M.W., Bethge, M., 2018. DeepLabCut: markerless pose estimation of user-defined body parts with deep learning. Nat. Neurosci. 21, 1281–1289. https://doi.org/10.1038/s41593-018-0209-y.

Medan, V., Preuss, T., 2014. The Mauthner-cell circuit of fish as a model system for startle plasticity. J. Physiol. 108, 129–140. https://doi.org/10.1016/j.jphysparis.2014.07.006.

Meserve, J.H., Nelson, J.C., Marsden, K.C., Hsu, J., Echeverry, F.A., Jain, R.A., Wolman, M.A., Pereda, A.E., Granato, M., Santistevan, N.J., Nelson, J.C., Ortiz, E.A., Miller, A.H., Halabi, D.K., Sippl, Z.A., Granato, M., Grinblat, Y., 2021. A forward genetic screen identifies Dolk as a regulator of startle magnitude through the potassium channel subunit Kv1.1. PLoS One 17, 1–28. https://doi.org/10.1371/journal.pone.0270903.

Miller, M.W., Gronfier, C., 2006. Diurnal variation of the startle reflex in relation to HPA-axis activity in humans. Psychophysiology 43, 297–301. https://doi.org/10.1111/j.1469-8986.2006.00400.x.

Nath, T., Mathis, A., Chen, A.C., Patel, A., Bethge, M., Mathis, M.W., 2019. Using DeepLabCut for 3D markerless pose estimation across species and behaviors. Nat. Protoc. 14, 2152–2176. https://doi.org/10.1038/s41596-019-0176-0.

O'Leary, T.P., Shin, S., Fertan, E., Dingle, R.N., Almuklass, A., Gunn, R.K., Yu, Z., Wang, J., Brown, R.E., 2017. Reduced acoustic startle response and peripheral hearing loss in the 5xFAD mouse model of Alzheimer's disease. Genes Brain Behav. 16, 554–563. https://doi.org/10.1111/gbb.12370.

Panlilio, J.M., Aluru, N., Hahn, M.E., 2020. Developmental neurotoxicity of the harmful algal bloom toxin domoic acid: cellular and molecular mechanisms underlying altered behavior in the zebrafish model. Environ. Health Perspect. 128, 1–19. https://doi.org/10.1289/EHP6652.

Preller, K.H., Ingold, N., Hulka, L.M., Vonmoos, M., Jenni, D., Baumgartner, M.R., Vollenweider, F.X., Quednow, B.B., 2013. Increased sensorimotor gating in recreational and dependent cocaine users is modulated by craving and attention-deficit/hyperactivity disorder symptoms. Biol. Psychiatry 73, 225–234. https://doi.org/10.1016/j.biopsych.2012.08.003.

Quednow, B.B., Kühn, K.U., Hoenig, K., Maier, W., Wagner, M., 2004. Prepulse inhibition and habituation of acoustic startle response in male MDMA ('Ecstasy') users, cannabis users, and healthy controls. Neuropsychopharmacology 29, 982–990. https://doi.org/10.1038/sj.npp.1300396.

Sandner, G., Canal, N.M., Brandão, M.L., 2002. Effects of ketamine and apomorphine on inferior colliculus and caudal pontine reticular nucleus evoked potentials during prepulse inhibition of the startle reflex in rats. Behav. Brain Res. 128, 161–168. https://doi.org/10.1016/S0166-4328(01)00273-X.

Santistevan, N.J., Nelson, J.C., Ortiz, E.A., Miller, A.H., Halabi, D.K., Sippl, Z.A., Granato, M., Grinblat, Y., 2022. Cacna2D3, a voltage-gated Calcium Channel subunit, functions in vertebrate habituation learning and the startle sensitivity threshold. PLoS One 17, 1–18. https://doi.org/10.1371/journal.pone.0270903.

Sato, K., 2020. Why is prepulse inhibition disrupted in schizophrenia? Med. Hypotheses 143, 109901. https://doi.org/10.1016/j.mehy.2020.109901.

Scaramella, C., Alzagatiti, J.B., Creighton, C., Mankatala, S., Licea, F., Winter, G.M., Emtage, J., Wisnieski, J.R., Salazar, L., Hussain, A., Lee, F.M., Mammootty, A., Mammootty, N., Aldujaili, A., Runnberg, K.A., Hernandez, D., Zimmerman-Thompson, T., Makwana, R., Rouvere, J., Tahmasebi, Z., Zavradyan, G., Campbell, C.S., Komaranchath, M., Carmona, J., Trevitt, J., Glanzman, D., Roberts, A.C., 2022. Bisphenol a Exposure Induces Sensory Processing Deficits in Larval Zebrafish during Neurodevelopment. eNeuro 9. https://doi.org/10.1523/ENEURO.0020-22.2022.

Siegelaar, S.E., Olff, M., Bour, L.J., Veelo, D., Zwinderman, A.H., Van Bruggen, G., De Vries, G.J., Raabe, S., Cupido, C., Koelman, J.H.T.M., Tijssen, M.A.J., 2006. The auditory startle response in post-traumatic stress disorder. Exp. Brain Res. 174, 1–6. https://doi.org/10.1007/s00221-006-0413-y.

Swerdlow, N.R., Geyer, M.A., 1998. Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. Schizophr. Bull. 24, 285–301. https://doi.org/10.1093/oxfordjournals.schbul.a033326.

Swerdlow, N.R., Auerbach, P., Monroe, S.M., Hartston, H., Geyer, M.A., Braff, D.L., 1993. Men are more inhibited than women by weak prepulses. Biol. Psychiatry 34, 253–260. https://doi.org/10.1016/0006-3223(93)90079-S.

Tagkalidou, N., Multisanti, C.R., Bleda, M.J., Bedrossiantz, J., Prats, E., Faggio, C., Barata, C., Raldúa, D., 2024. Analyzing the effects of age, time of day, and experiment on the basal locomotor activity and light-off visual motor response assays in zebrafish larvae. Toxics 12. https://doi.org/10.3390/toxics12050349.

Wolman, M.A., Jain, R.A., Liss, L., Granato, M., 2011. Chemical modulation of memory formation in larval zebrafish. Proc. Natl. Acad. Sci. 108, 15468–15473.

Wolman, M.A., Jain, R.A., Marsden, K.C., Bell, H., Skinner, J., Hayer, K.E., Hogenesch, J. B., Granato, M., 2015. A genome-wide screen identifies PAPP-AA-mediated IGFR signaling as a novel regulator of habituation learning. Neuron 85, 1200–1211. https://doi.org/10.1016/J.NEURON.2015.02.025/ATTACHMENT/0B828D8E-3735-4D5C-BD87-0CD9DC4CCBA1/MMC4.MP4.