Maintenance of Blood Pressure in Emotional Context-based Autonomic Switching by the Central Nucleus of the Amygdala in Rats

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Abstract

Proper autonomic control is necessary in making appropriate decisions and actions, but neuronal mechanisms for this function are yet to be determined. Here we show that the amygdala plays a role in autonomic cardiovascular tuning in a dynamically changing environment. We recorded blood pressure and heart rate of head-restrained rats during appetitive and aversive classical conditioning tasks. Rats learned varying associations between conditioned stimuli and unconditioned stimuli in three types of contexts: appetitive, neutral, and aversive blocks. Blood pressure and heart rate in the appetitive block gradually increased after reward-predicting cues, followed by a vigorously increased response to the actual reward. The predictive response was found to be significantly higher than the responses in the neutral and aversive blocks. Blood pressure and heart rate responses to the air puff-predicting cue in the aversive block were significantly lower than that of the responses in the neutral block. Pharmacological blockade of the amygdala has significantly decreased reward-predictive pressor responses in the latter phase, but not in the initial phase of context change. Cardiovascular responses are thus adaptively tuned by positive and negative emotional stimuli, and the central nucleus of the amygdala likely assists in maintaining pressor response tuning based on emotional context.

Introduction

Predicting outcomes and preparing actions for future events based on current contexts can often result in getting rewards ahead of competitors and flight from danger. Such prediction is critical for survival, especially in a dynamically changing environment. Motor control is essential for appropriate and rapid action, and autonomic cardiovascular tuning is likewise critical to supply energy to active skeletal muscles. However, the neuronal mechanisms underlying dynamic adjustments of autonomic cardiovascular responses are still unclear.

Previous studies show that the amygdala plays an essential role in emotional processing and defensive behavioral responses. The amygdala is classically thought to play a central role in a negative emotional aspect[1]; however, nuclei involvement in emotional, attentional, and learning processing from a positive aspect has also been reported in rodents[2-6], macaques[7,8], and humans[9]. Shabel and Janak show that the neuronal activities of the amygdala during appetitive and aversive conditioned stimuli are similar and correlated with autonomic arousal, such as changes of blood pressure, suggesting an emotional arousal coding in this brain region[10,11]. We showed that electrical and chemical stimulation of the central nucleus of the amygdala (CeA) in anesthetized rats induced bidirectional (facilitatory or inhibitory) cardiovascular responses in a region-specific manner, indicating that the amygdala displays the neuronal circuitry for modulating autonomic responses[12]. In fact, high-intensity treadmill running with negative emotion has caused a sudden increase in blood pressure immediately before exhaustion and strong c-Fos expression in the amygdala[13]. Conditioned cardiovascular responses are classically recorded during anticipation of either appetitive or aversive outcomes[14,15]. Because many of these studies have been conducted in free-moving animals, however, it is difficult to control animal's behaviors. Furthermore, when facing an

environment where emotional context dynamically changes, autonomic cardiovascular response is also dynamically regulated, and amygdala involvement has not been demonstrated.

Thus, we hypothesized in this study that the amygdala plays a role in autonomic cardiovascular tuning in accordance with the context of emotional arousal. In our experiments, we recorded blood pressure and heart rate from head-restrained rats to test the hypothesis during appetitive and aversive classical conditioning tasks and examined the effects of amygdala manipulation.

Results

Blood pressure and heart rate responses during classical conditioning

We developed a classical conditioning task with context switching (Fig. 1) to investigate mechanisms underlying cardiovascular regulation while dynamically changing emotional contexts. Head-restrained rats with pre-implanted radio transmitters (n = 7) were trained to discriminate between two tone cues during appetitive reward (RW), neutral (NA), and aversive (AV) contexts. One tone (CS+, 10 kHz) cue was associated with reward delivery (reward CS+ predicts 5% sucrose, 0.08 mL, reward US+) in the RW context, no outcome in the NA context (neutral CS+), and punishment in the AV context (aversive CS+ predicts an air puff, 30–40 psi, 1 s, aversive US+). The other tone (CS−, 4 kHz) cue was associated with no outcome in all contexts (Fig. 1b, reward, neutral, and aversive CS−). We used data after sufficient training as following analysis.

Example blood pressure and heart rate traces in two trials in RW and AV contexts are provided in Figure 2a and 2b. Blood pressure and heart rate in the RW context have been determined to gradually increase during the CS–US interval, followed by a phasic pressor and vigorous tachycardiac response to the reward US+, but not reward CS− and US− (Fig. 2a). Conversely, blood pressure in the AV blocks showed phasic depressive responses to both aversive CS+ and US+. Still, heart rate was observed to decrease after aversive CS+, and phasic tachycardiac responses occurred after aversive US+ (Fig. 2b) compared to responses in the NA context.

Averaged blood pressure responses compared with baseline activity (ΔBP) to reward CS+ (+7.13 ± 0.17 mmHg) and aversive CS+ (−0.05 ± 0.10 mmHg) were significantly higher and lower than that of the neutral CS+ (+1.70 ± 0.14 mmHg), respectively (Fig. 2c, $F_{(2,5567)}$ = 744.9, p < 0.001, one-way ANOVA with Bonferroni's post hoc test). Similarly, heart rate response compared with baseline activity (ΔHR) to reward and aversive CS+ (RW: 23.29 ± 0.66 bpm, AV: 11.77 ± 0.45 bpm) was significantly higher and lower, respectively, than in the responses in NA contexts (15.53 ± 0.51 bpm) (Fig. 2d, $F_{(2,5572)}$ = 114.58, p < 0.001). Interestingly, ΔBP and ΔHR for reward CS− (0.70 ± 0.14 mmHg and 6.09 ± 0.54 bpm) showed significantly increasing and decreasing responses compared to NA (−0.42 ± 0.09 mmHg and 9.23 ± 0.46 bpm) and AV (−0.66 ± 0.09 mmHg and 7.35 ± 0.44 bpm) during the CS–US interval (Fig. 2e, $F_{(2,5627)}$ = 44.60, p < 0.001; Fig.2f, $F_{(2,5635)}$ = 10.49, p < 0.001), even though CS− (4 kHz tone) indicated no outcome in all trials throughout the classical conditioning.

Dynamic changes in cardiovascular responses triggered by emotional context switching

We then assessed context-dependent cardiovascular responses during the CS–US interval using our dynamically changing appetitive and aversive classical conditioning task. Pseudocolor plots of averaged ΔBP in response to CS+ and CS− in five trials before and after context switching (Fig.3a, NA→RW→NA; Fig.3b, NA→AV→NA) indicate that unanticipated context switching from neutral to reward contexts (NA→RW) produced ΔBP to reward CS+ (−1.38 ± 0.31 mmHg, p = 0.42, Mann-Whitney U test) similar to responses in the previous NA context block (−1.13 ± 0.16 mmHg). However, once receiving reward US+, responses to reward CS+ were dramatically increased (4.38 ± 0.42 mmHg, p < 0.001, Mann-Whitney U test). Pressor responses to reward CS+ were maintained until a few trials after switching back to the neutral block (RW→NA): 4.57 ± 0.26 mmHg in five trials before context switching, 3.93 ± 0.44 mmHg in first trials (p < 0.001, compared to ΔBP to CS+ in NA context), 1.02 ± 0.35 mmHg in second trials (p < 0.001), −0.16 ± 0.31 mmHg in third trials (p = 0.005), and −0.75 ± 0.26 mmHg in fourth trials (p = 0.52) after context switching. Immediately after the transition from neutral to aversive contexts (NA→AV), slightly but significant depressor response to aversive CS+ (−0.66 ± 0.14 mmHg in five trials before context switching, −1.06 ± 0.29 mmHg in first trials, p = 0.06, −1.20 ± 0.24 mmHg in second trials, p < 0.001) and prominent tri-phasic responses (increase–decrease–increase) to aversive US+ were observed (Fig. 3b). ΔBP showed a consistent phasic depressor response to CS− and pressor response to US− regardless of context, but the US response disappeared only in RW context blocks. ΔHR to CS showed similar response characteristics as ΔBP (Fig. 3c-d). Context transition between NA→RW and RW→NA required two or three trials in order for heart rate to switch. Cardiovascular responses to reward- and aversive-predictive cues rapidly changed, guided by reward and aversive expectations.

Bilateral inactivation of the CeA attenuated reward prediction-induced pressor response

We used classical conditioning to evaluate the cardiovascular regulation based on emotional contexts. Previously, we reported that microstimulation of the CeA induces site-specific, bidirectional (pressor/tachycardiac and depressor) cardiovascular responses in anesthetized rats, suggesting possible involvement in autonomic tuning[12], through supporting the expression of defensive behaviors in emergency situations. If the CeA plays a causal role in the regulation of blood pressure during emotional arousal, inactivation of CeA should abolish responses to emotional CSs.

We then examined the effects of cardiovascular responses to CSs to test this possibility via inactivation of bilateral CeA (1.8 mm caudal to bregma, 3.0 mm lateral to midline, and 7.0 mm ventral to dura). We used four of the seven rats in the experiment. The $GABA_A$ receptor agonist muscimol (80 pmol, 100 nL) was microinjected while the rats performed the conditioning task. The injection site was confirmed using fluorescent microspheres (FluoSpheres, 100 nL) based on the histology (Fig. 4, right panel). In total, we performed 23 injections (muscimol, 12 injections; saline, 11 injections) in 4 rats. All animals were injected with both muscimol and saline on different sessions. Inactivation of bilateral CeA using muscimol compared with saline injection caused significant decreases in ΔBP before and after switching from the RW to NA context blocks ($F_{(15,1287)}$ = 2.64, P < 0.006, significant interaction, two-way ANOVA). Notably, the

decrease in ΔBP was observed during the second half of the RW context block ($p < 0.001$) but not immediately after NA→RW switching ($p > 0.05$, Mann–Whitney U test). CeA likely plays a more important role in maintaining rather than in acquiring emotional responses to salient events.

Discussion

Autonomic cardiovascular responses of head-fixed rats show characteristic activity depending on appetitive, neutral, and aversive contexts. Switching contexts provokes rapid regulation in accordance to emotional contexts. Furthermore, blockade of the activity of the CeA impaired pressor responses during switching from reward to neutral contexts. We demonstrate that cardiovascular responses are adaptively and rapidly tuned by positive and negative emotional stimuli, and CeA may contribute to the maintenance of adaptive responses.

We confirmed the characteristic cardiovascular responses in our classical conditioning task. Blood pressure exhibits a phasic and gradual increase in response to reward-predictive cues and a phasic decrease to neutral- and aversive-predictive cues. Heart rate increased in response to cues under all conditions, but the level of tachycardia varied among conditions: CS+, in the RW context, induced predominant tachycardia, while in the AV context, a relatively small increase of heart rate was evoked compared to the NA context. Previous studies have reported cardiovascular responses during classical conditioning task using free-moving and restrained animals in several species, such as pigeon[16], rabbit[17], dog[18-20], rodent[10,11,21,22], marmoset[23,24], macaque[25], and humans[26,27]. Appetitive conditioning using food or water as a reward US, induces pressor and tachycardia during the CS–US interval, while, in aversive contexts, using electrical shock as punishment US, often causes pressor or tachycardia, but some bradycardic responses are reported[14,15,25]. Small but significant depressive and lower heart rate responses to air puff-predicting CS+ were also observed. Air puff is definitely acting as the US based on the remarkable tri-phasic cardiovascular responses. Possible reasons that air puff-predicting CS+ caused suppression but not facilitation of cardiovascular responses include overtraining of Pavlovian procedures where animals cannot avoid punishment (e.g., learned helplessness) or the air puff might be a weak stimulus compared with electrical shock.

Interestingly, only in the RW context, CS− and US− were observed to induce relatively higher blood pressure and lower heart rate responses, even though CS− was not associated with either reward or punishment in any contexts. Animals could get rewarded in our task after CS+ only in the RW context. The RW block with the highest value CS+ represents higher state value, and CS− in the context may induce stronger negative emotion compared with negative impacts in other contexts. This possibility suggests that the value of an option (CS−) is assigned relatively, rather than absolutely, and is often influenced by the value of the paired option (CS+).

The roles of the amygdala in classical conditioning have already been examined in previous studies, particularly through fear conditioning in free-moving animals. The CeA projects into various regions such as the lateral hypothalamus and the brainstem to regulate autonomic sympathetic and parasympathetic

activities and endocrine responses to promote defensive behaviors (attack, escape, and freeze)[28]. Lesions in the bilateral amygdala attenuated conditioned acceleration of heart rate in both appetitive[23] and aversive[24,29,30] conditioning using free-moving animals. These findings suggest that the amygdala may be involved in the autonomic arousal in emotional processing. Many studies examined either appetitive or aversive context, little is known concerning autonomic tuning in situations where emotional context changes dynamically between appetitive and aversive. Our results are also consistent with previous studies in which the amygdala (CeA) plays an important role in accelerating cardiovascular tuning. Notably, the attenuation of pressor responses by amygdala inactivation did not appear immediately after switching to the appetitive (RW) context but appeared when switching from the appetitive to neutral (RW→NA) context, suggesting a more crucial role in the maintenance, than acquisition, of emotional autonomic tuning.

Lack of significant changes in cardiovascular responses to aversive-predicting cue by amygdala inactivation is a limitation in this study. Conditioned responses to fear-predictive stimuli are task-dependent[14], and decelerated responses observed in our study are not that strong. Additionally, based on previous electrophysiological studies, many amygdala neurons encode emotional arousal that responds to both appetitive- and aversive-predicting CS[10,11]. Blockade of the amygdala may diminish autonomic responses if accelerated or more decelerated responses were evoked by aversive CS.

Our findings demonstrated cardiovascular tuning and the involvement of the CeA in dynamically changing emotional contexts. Context-dependent cardiovascular response rapidly adapts in a few trials just after context switching. Such rapid switching is reminiscent of the activity of neurons of the amygdala[31], striatum[32], and midbrain dopamine neurons[33]. Blockade of dopamine D1 receptor in the striatum impaired context-dependent behavioral responses[34,35]. Artificial suppression of the midbrain dopaminergic neurons during fear-to-safety context switching induced a delay in the extinction of fear conditioning behavior[36]. In addition, the activity of dopamine neurons has also been reported to contribute to the prediction of reward as well as aversion stimuli[37,38]. Anatomically, it is known that the amygdala also has received dopaminergic inputs[39]. There is a direct projection from the amygdala (basolateral region) to the striatum, while projection from the striatum to the amygdala is thought to be polysynaptic. Functionally, activities of the striatal neurons increased in response to reward-predicting cue, but their activities were attenuated by the blockade of the amygdala[40]. Based on these evidences, the context-dependent cardiovascular responses we observed in the present study may reflect differences in outcome expectation with the functional interactions between the amygdala and the striatum via dopaminergic inputs[41]. Thus, future studies should further examine the functions of neuronal circuits that may support and maintain emotional expression suitable for a dynamically changing environment.

Methods

Animals

Totally, seven male Wistar–Kyoto rats (7 weeks old, 235 ± 44 g at the time of first surgery) were used in this study (Japan SLC). The animals were housed in a temperature-controlled room under a fixed 12:12 h dark/light cycle (6:00–18:00/18:00–6:00). Animals were provided access to food and water *ad libitum* after recovery from surgery. Water access was restricted during behavioral learning of task to increase motivation for sucrose rewards. Body weights of rats were measured every day and given a few agar blocks (containing 15 mL water) in their home cages when they were less than 85–90 % of their original weights. All experiments were approved by the Ethics Committee for Animal Experiments at Juntendo University and complied with the guidelines set by the Japan Physiological Society.

Surgery

We implanted radio transmitter to record blood pressure and then head plates to fix animal's body during experiments. Recovery time after each surgery was over 1 week. During surgery, rectal temperature was monitored and maintained at 37 °C using a heating pad (BWT-100, Bio Research Center, Japan). The level of anesthesia was checked by assessing limb withdrawal to noxious pinching. After surgery, antibiotics (benzylpenicillin, 1000U, i.m., Meiji Seika Pharma, Japan) and analgesics (meloxicam, 1mg/kg, s.c., Boehringer Ingelheim, Germany) were administered.

Implantation of a transmitter for telemetry

A telemetric radio transmitter (HD-S10; Data Sciences International, USA) for chronically blood pressure recording from abdominal aorta was implanted as described previously[12,42]. Rats were anesthetized with pentobarbital sodium (50 mg/kg) by intraperitoneal (i.p.) administration and isoflurane (2.0–2.5 % for maintenance) using an inhalation anesthesia apparatus (Univentor 400 anesthesia unit, Univentor, Sweden). After an abdominal midline incision was made in a rat in supine position, the intestines were moved aside to allow visualization, and the abdominal aorta was carefully isolated. After a temporal blockade using a sterilized string to prevent severe blood loss, the tip of the catheter of the transmitter was inserted into the abdominal aorta along a 21G needle guide. The transmitter catheter was then fixed using a tissue adhesive (Vetbond, 3M, USA). The transmitter was sutured to the ventral wall of the abdominal cavity.

Head-fixed operation

All procedures for the head plate implantation (CFR-1, Narishige, Japan) were referenced to previously established studies[43-45]. Rats were anesthetized with isoflurane (Pfizer, USA), 4.5–5.0 % for induction and 2.0–2.5 % for maintenance, and they were later placed on a stereotaxic frame (SR-10R-HT, Narishige, Japan). A stainless head plate was then attached to the skull using tiny stainless screw bolts (M1, 2 mm long; Yahata Neji Corporation, Japan) as anchors along with dental cements (Super-Bond C&B, Sun Medical; Unifast II, GC Corporation, Japan).

Classical conditioning task

Rats were trained using the behavioral testing systems (Task Forcer, O'Hara and Co.,Ltd., Japan) with classical conditioning in three types of contexts (Fig. 1); this is to develop a behavioral model for evaluation of autonomic responses during dynamic changing environments with emotional contexts. Animals were fixed on a stereotaxic frame (SR-10R-HT, Narishige, Japan) in a sound-attenuated box (SAC-4201W, O'Hara and Co.,Ltd., Japan) and learned varying associations between conditioned stimuli (CS) and unconditioned stimuli (US) in three contexts (Figs. 1a and 1b): (1) Appetitive reward (RW) context: one tone cue (reward CS+, 10 kHz, 1 s) predicted sucrose delivery (reward US+; 5% sucrose, 0.08 mL), and another tone cue (reward CS−, 4 kHz, 1 s) predicted non-reward (reward US−), (2) Aversive (AV) context: the CS+ predicted air puff (aversive US+; 30–40 psi, 1 s) and the CS− predicted no air puff (aversive US−), (3) Neutral (NA) context: both CS tones predicted nothing (neutral CS+ and CS−). The air puff was delivered through a stainless tube placed 8–10 cm from the rat's face. The interval of the time between CS offset to US onset (CS–US interval) was 15 s. The inter-trial interval was 60 ± 15 s. Each block consisted of 16 to 24 trials, and the order of trials (CS+ or CS− trial) was pseudo-randomly assigned. Cues did not occur with context changes, and animals could not predict the timing of context block switching. The order of context blocks was fixed as RW and AV were alternatively presented; NA was then deployed in between (Fig. 1c; RW → NA → AV → NA → RW →…). RW or AV was randomly assigned to start the daily trials. As a learning process, rats were initially trained only in the RW context. The AV context was then added, with NA context training included last.

Muscimol injection

Causality between context-based blood pressure responses and activity of the CeA was assessed using pharmacological inactivation experiments. Four of the seven animals were used in this experiment. Before initiating the classical conditioning, rats were microinjected with $GABA_A$ receptor agonist (muscimol, 80 pmol, 100 nL, M1523-10MG, Sigma-Aldrich, USA) into the bilateral CeA (1.8 mm caudal, 3.0 mm lateral from bregma, and 7.0 mm ventral from dura) using a glass micropipette (outside diameter of 20–30 μm; GC200F-10, Harvard Apparatus, USA). We also used saline injection (100 nL, Otsuka, Japan) to control for cardiovascular responses due to volume effects of liquid injection. Micropipettes were then connected to a Hamilton microsyringe mounted on a syringe pump (LEGATO110, KD Scientific, USA) to control the injection rate (500 nL/min). After completing the final experiments, identification of the chemical inactivation site was confirmed by injections of fluorescent microspheres (FluoSpheres, 100 nL, Thermo Fisher Scientific, USA) at the same coordinates stereotaxically as muscimol injection.

Histology

Rats were deeply anesthetized with sodium pentobarbital and isoflurane after completion of all experiments and intracardially perfused with saline followed by 4 % paraformaldehyde (163-20145, FUJIFILM Wako Pure Chemical Corporation, Japan). The brains were then removed, post-fixed for at least 48 h in 4 % paraformaldehyde, and replaced with 30 % sucrose. Brain tissue that settled out in the sucrose solution was sliced into 50-μm-thick serial sections on a freezing microtome (REM-710; Yamato Kohki Industrial, Japan). The sections were then mounted on slides and imaged using a fluorescence

microscope (EVOS FL Auto 2 imaging system, Thermo Fisher, USA) to map drug injection sites in the amygdala.

Data analysis and statistics

We recorded blood pressure and heart rate during the classical conditioning tasks. These parameters were simultaneously monitored and recorded using the telemetry blood pressure recording system (PhysioTel, Data Sciences International, USA) with the PowerLab system (PowerLab/8s, ADInstruments, New Zealand). Mean blood pressure and heart rate were derived from pulsatile pressure signals using LabChart software (Version 8.0, AD Instruments). These data were subsequently analyzed in MATLAB (The MathWorks, USA). Artificial drops or increases in blood pressure and heart rate signals were removed and treated as missing values in the dataset. We mainly focused on our analysis on blood pressure and heart rate from CS onset to US onset (CS–US interval), and changes in blood pressure (Δblood pressure) and heart rate (Δheart rate) were calculated by subtracting mean values during baseline period 5–15 s before the CS onset. One-way analysis of variance (ANOVA) with Bonferroni's post hoc test was used in comparing magnitudes of blood pressure and heart rate among RW, NA, and AV contexts. Analysis of blood pressure and heart rate with switching of the emotional context was calculated using ensemble responses to each CS during five trials before and after block switch. Finally, we quantified and compared CS–US intervals between muscimol-and saline-injected sessions to examine the effects of inactivation of the CeA. We then analyzed the data using two-way ANOVA and Mann–Whitney U tests with trials from context change and drug (muscimol vs saline) factors. Statistics analysis was conducted using MATLAB Statistics and Machine Learning Toolbox (The MathWorks). The criterion for statistical significance was $p < 0.05$.

Declarations

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Author contributions

K.Y. and H.W. designed the research; K.Y. conducted the experiments; K.Y. analyzed the data; and K.Y. and H.W. wrote and reviewed the manuscript.

Competing interests

The authors declare no competing financial interests.

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Figures

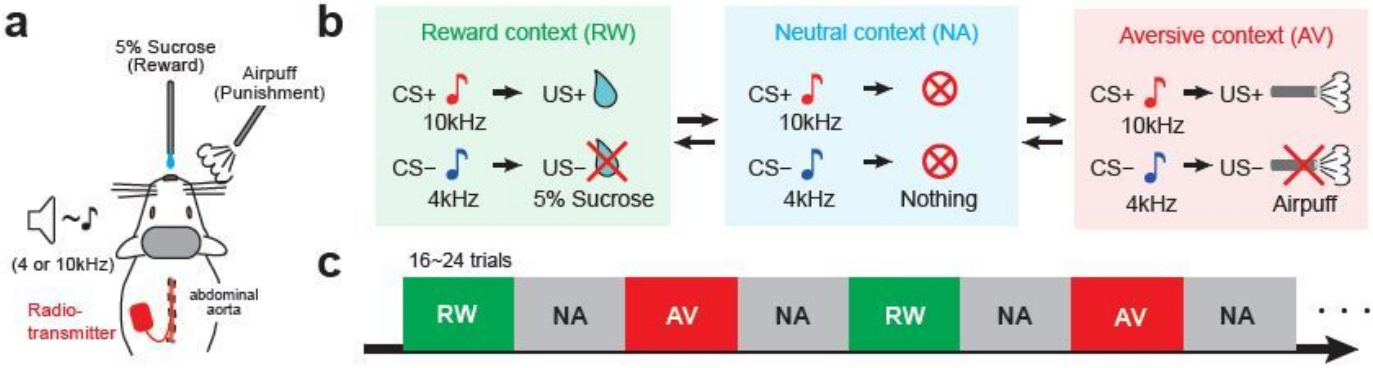
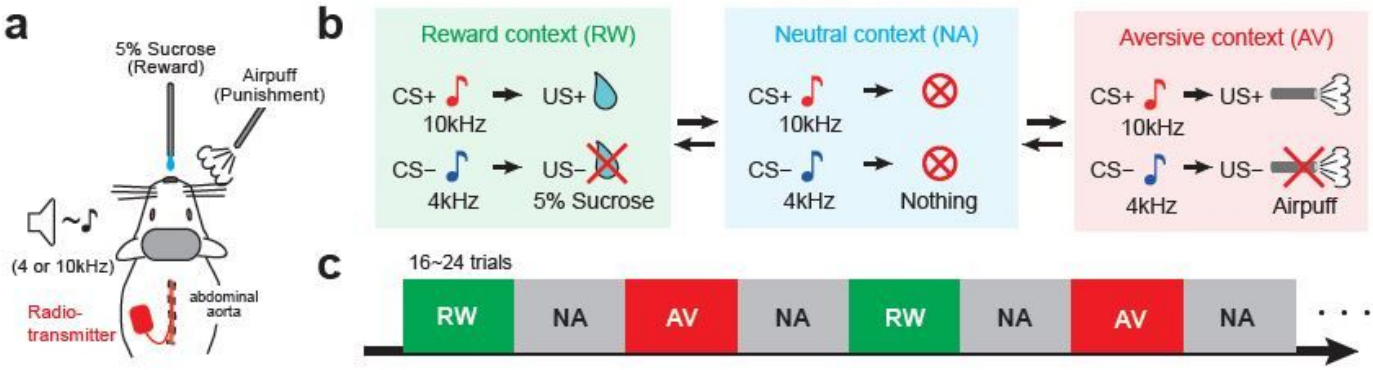


Figure 1

Pavlovian conditioning task with emotional valence. (a) Schematic diagram of the behavioral task. Stainless tubes for reward and air puff delivery were placed in front of the head-fixed rat's face. A radio transmitter was implanted into the abdominal aorta to record blood pressure by telemetry. (b) Pavlovian procedure with three distinct contexts. A tone cue (CS+, 10 kHz) preceded reward (US+, 5% sucrose), and another cue (CS−, 4 kHz) preceded non-reward (US−) in the reward (RW) blocks; CS+ preceded an air puff (US+) and the CS− preceded no-air puff (US−) in the aversive (AVE) blocks; both CS tones preceded nothing in the neutral (NA) blocks. CS, conditioned stimulus; US, unconditioned stimulus. (c) Trial sequence of the behavioral task. NA blocks were alternately deployed between RW and AV blocks. There were 16 to 24 trials per block. The order of blocks was fixed, but whether it started with RW or AV was randomized for every experimental day. There was no sign when changing blocks.



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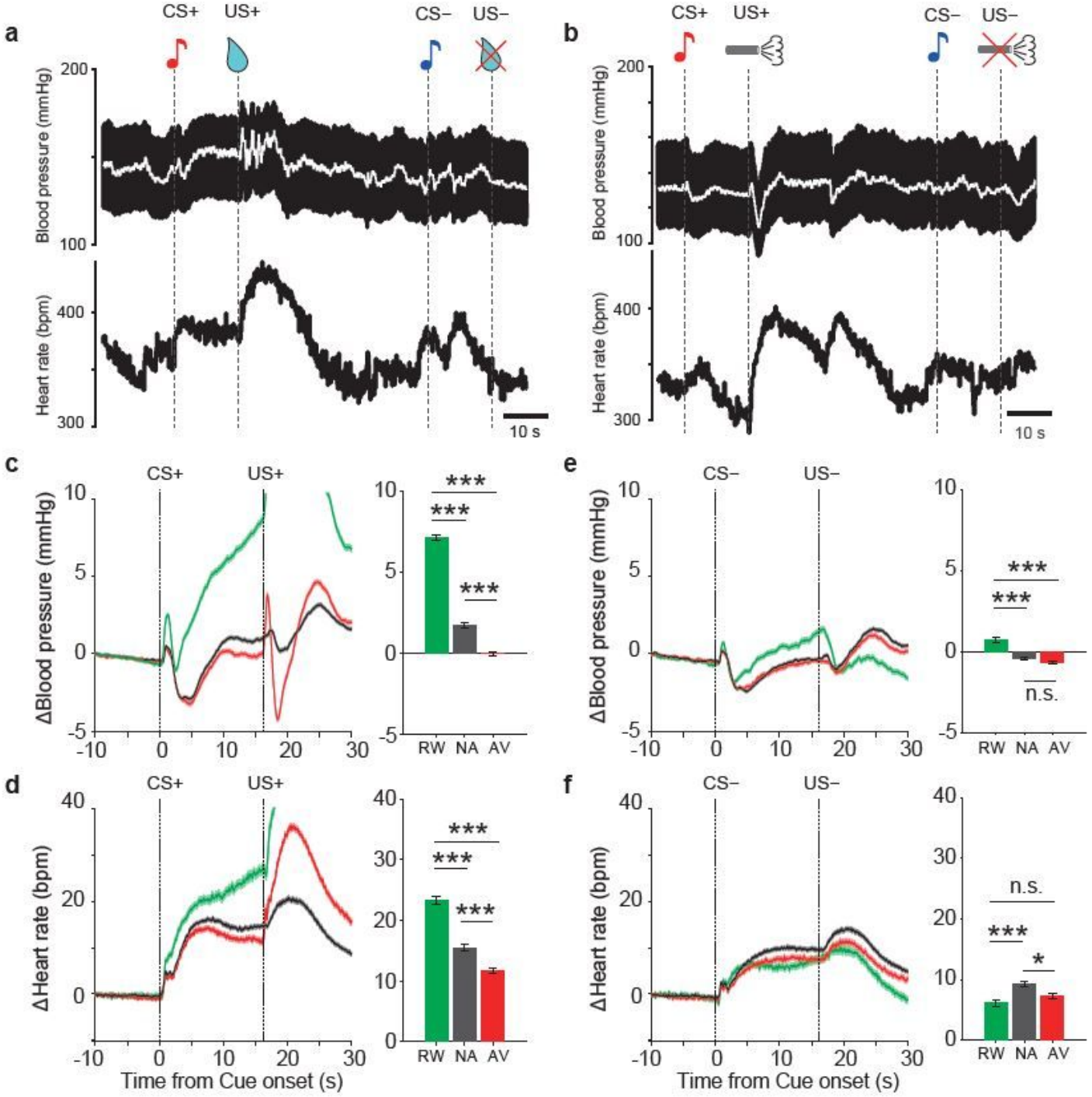
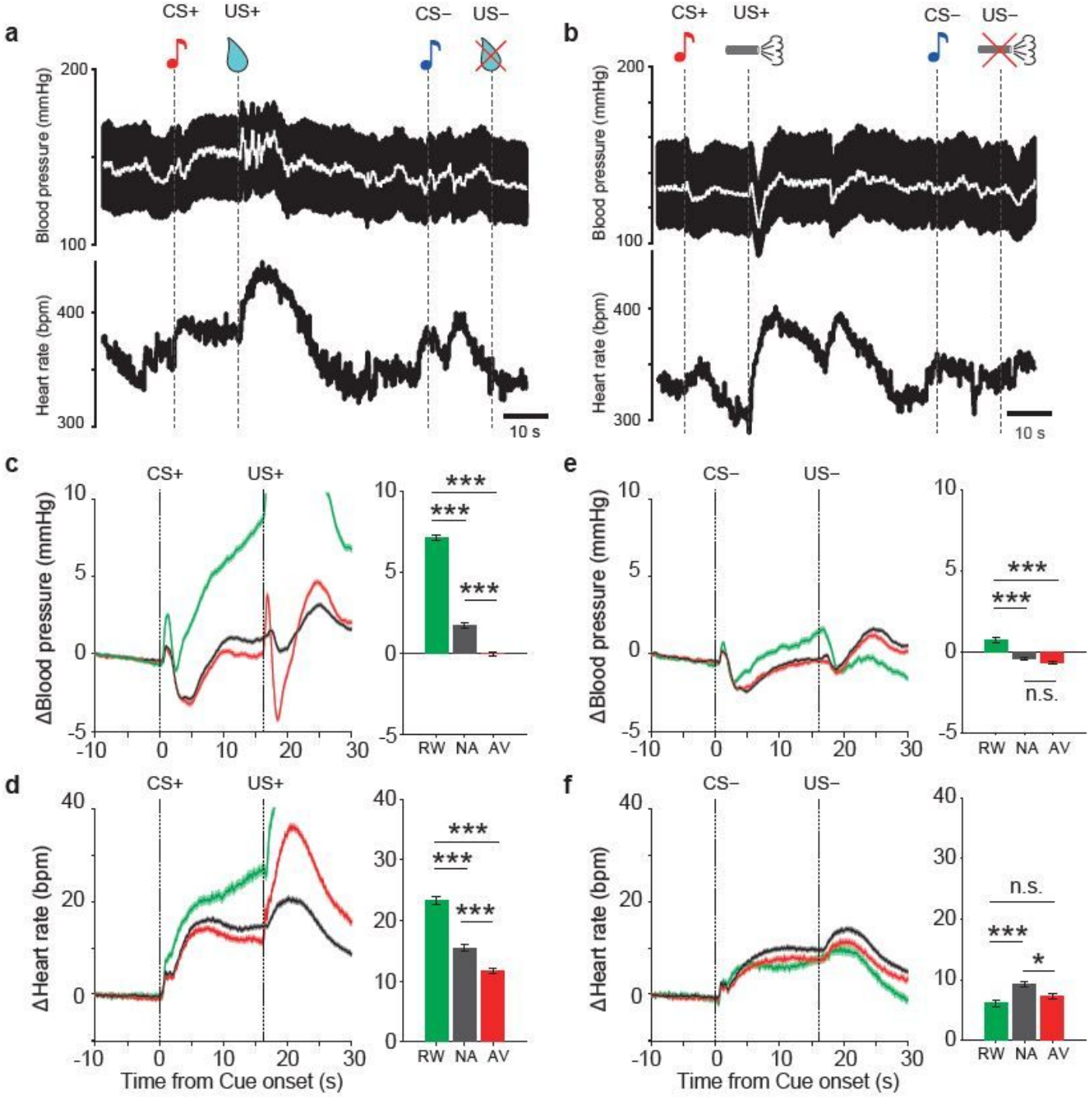


Figure 2

Blood pressure and heart rate responses during the Pavlovian conditioning task. (a, b) Example traces in two trials during the RW block (a) and AV block (b). Dotted vertical lines indicate the timing of CS or US onset. (c, d) Averaged responses of Δblood pressure (c) and Δheart rate (d) responses to CS+ and US+ during the RW (green line and bar), NA (black line and bar), and AV (red line and bar) blocks. (e, f) Same as (c, d), but during CS− and US−. **p < 0.01, *** p < 0.001, one-way ANOVA with Bonferroni's post hoc test.



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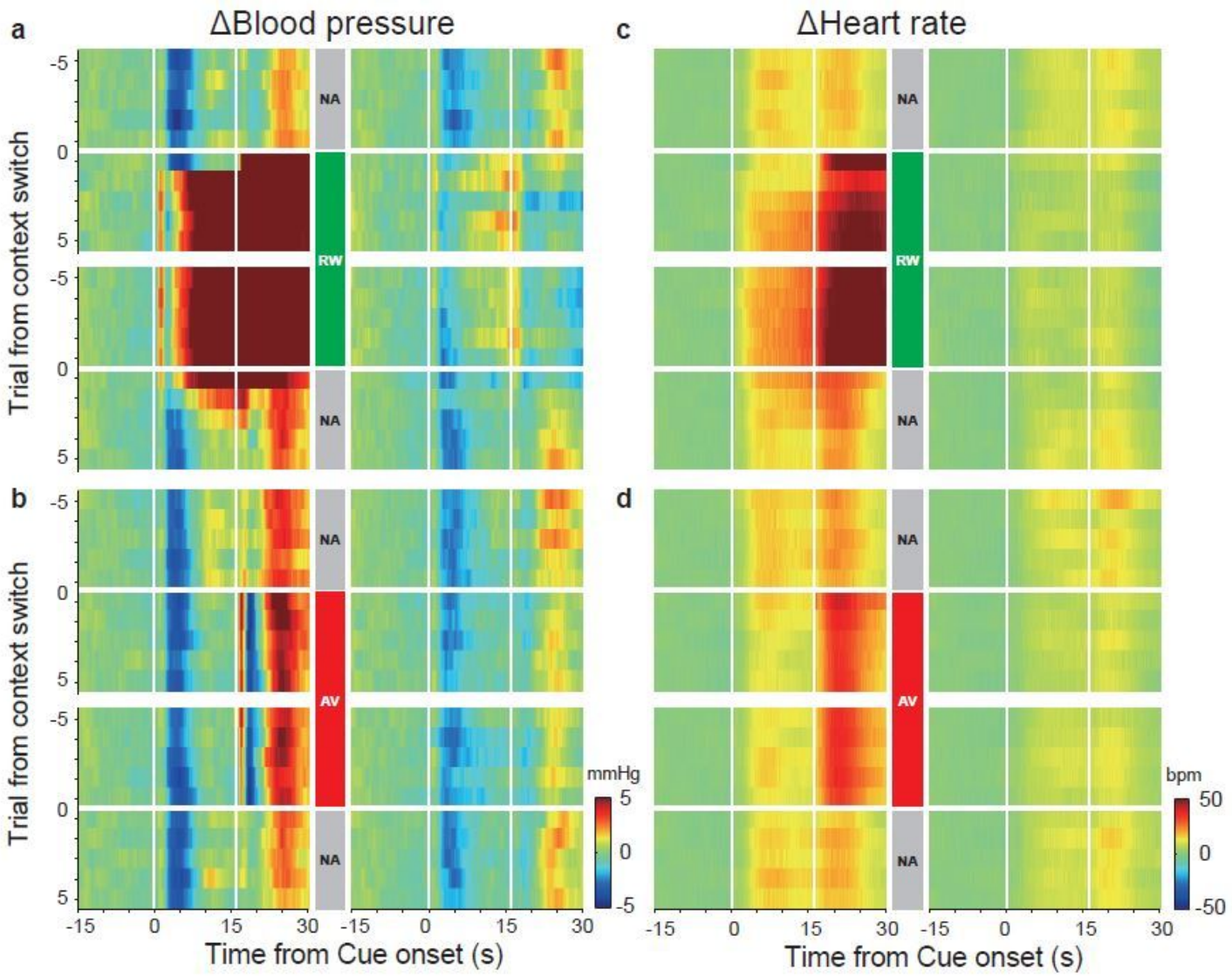
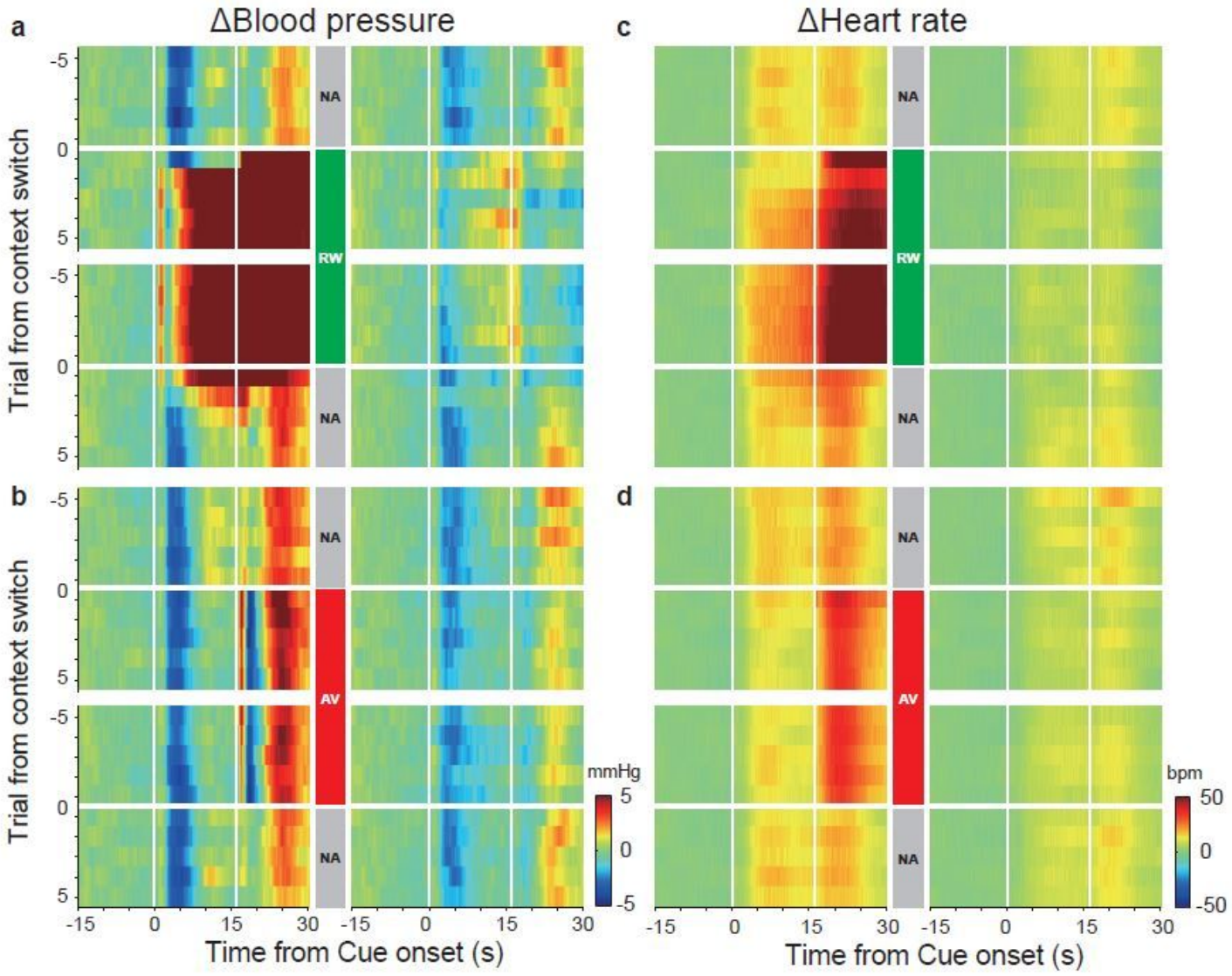


Figure 3

Dynamic changes of blood pressure responses triggered by emotional context switching. (a, b) Pseudo-color plots showing averaged Δblood pressure during five trials in each trial type CSs (left panels, CS+; right panels, CS−) before and after switching of reward context (a, NA→RW, RW→NA, from upper to bottom) and aversive context (b, NA→AV, AV→NA, from upper to bottom). (c, d) Same as (a, b) but Δheart rate.



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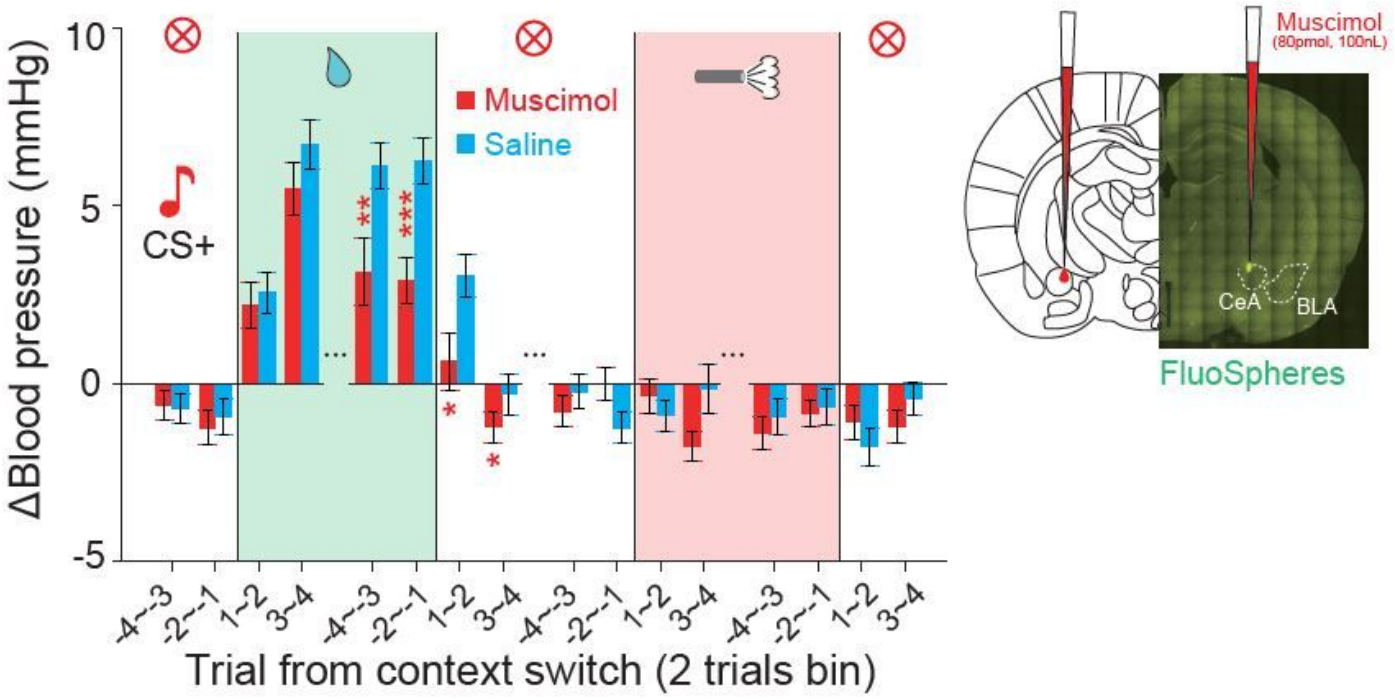
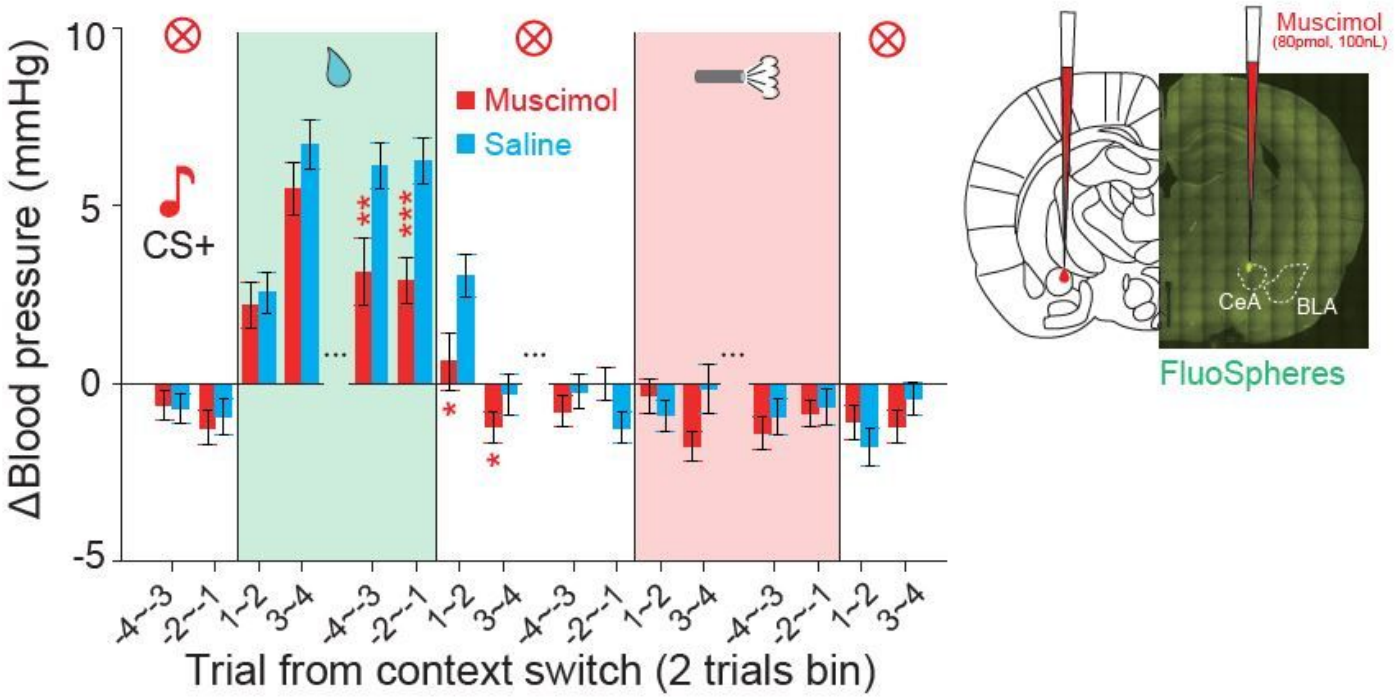


Figure 4

Bilateral inactivation of the CeA attenuated reward prediction-induced pressor response. Average data of Δblood pressure in response to CS+ for the effects of bilateral inactivation of the CeA before and after four trials (two trials per bin) at context switching during the Pavlovian conditioning task (left panel). Red and cyan bars indicate the data of muscimol (n = 12) and saline (n = 11). *p < 0.05, **p < 0.01, ***p < 0.001, two-way ANOVA and Mann–Whitney U test. The injection site of a GABAA agonist, muscimol (80 pmol. 100 nL), in the bilateral CeA. Fluorospheres (100 nL) were injected at stereotaxically identical positions using glass micro pipettes (right panel).



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