

Responding to social and non-social challenges: the modulating roles of 2-AG and corticosterone

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Introduction

Previous studies showed that the behavioral effects of enhanced 2-arachidonoyl-glycerol (2-AG) signaling induced by monoacilglycerol-lipase (MAGL) blockade highly depend on environmental conditions e.g. aversiveness of testing context. As the hypothalamus-hypophysis-adrenal-axis (HPA-axis) is differentially activated in differentially aversive situations, we studied the possible involvement of the axis in MAGL blockade-induced behavioral changes. As behavioral effects of endocannabinoid manipulations are reported to differ in social and non-social challenges we also aimed to study how enhanced 2-AG signaling alters behavioral and neuroendocrinological responses in social and non-social paradigms, respectively.

Effects of MAGL blockade on behavioral and endocrine responses to non-social challenges

We investigated the effects of the MAGL enzyme blockade induced enhancement of 2-AG signaling on HPA axis activity and behaviour. In the elevated plus maze (**Fig.1-3.**) and open field tests (**Fig.4.**) we co-administrated corticosterone synthesis inhibitor Metyrapone to study the CORT dependence of behavioural effects.

HPA-AXIS ACTIVITY

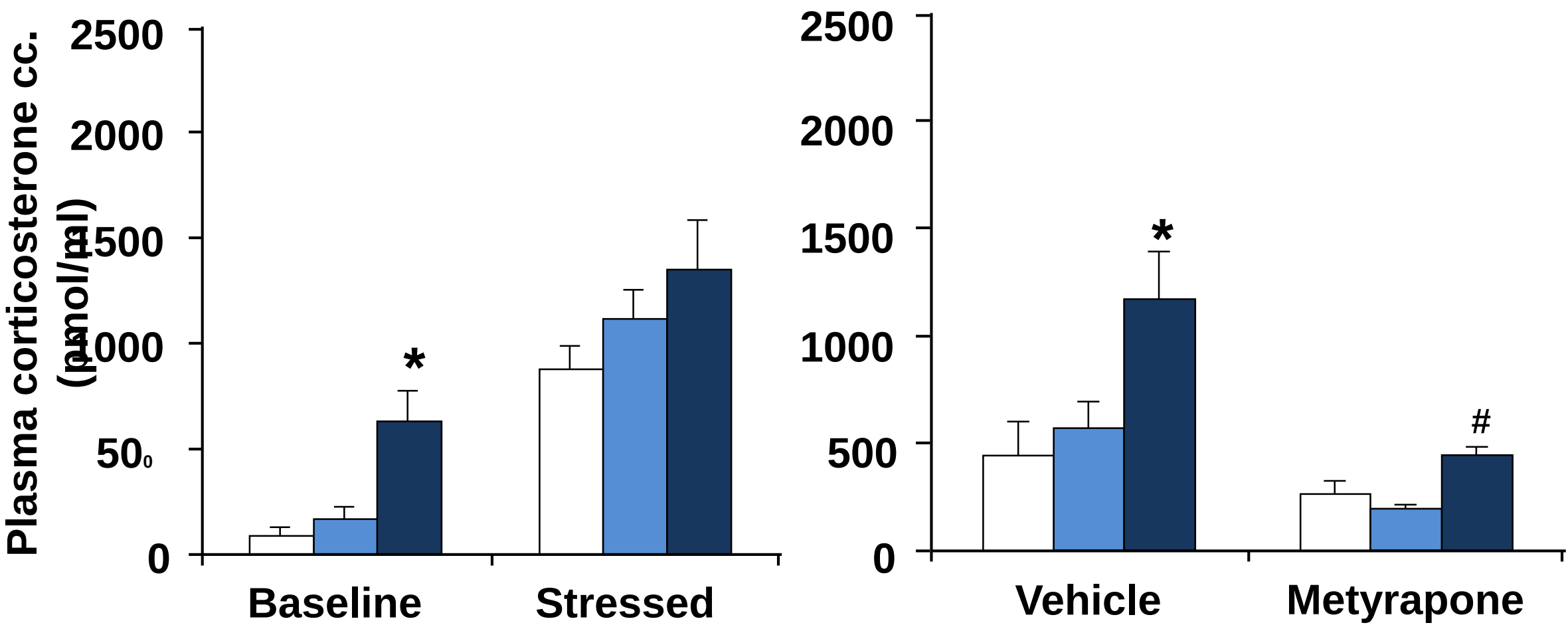


Fig.1. Effects of the enhanced 2-AG signaling on the corticosterone synthesis 40 min after treatment. JZL184 induced MAGL blockade significantly and rapidly increased basal CORT levels in a dose dependent manner, while left the stress-induced levels intact. Metyrapone administration drastically decreased CORT synthesis.

□ Vehicle ■ 8 mg/kg JZL184 ■ 16 mg/kg JZL184

*: significant difference from the other JZL184 treated groups. #: significant difference from the groups treated only with JZL184

ANXIETY

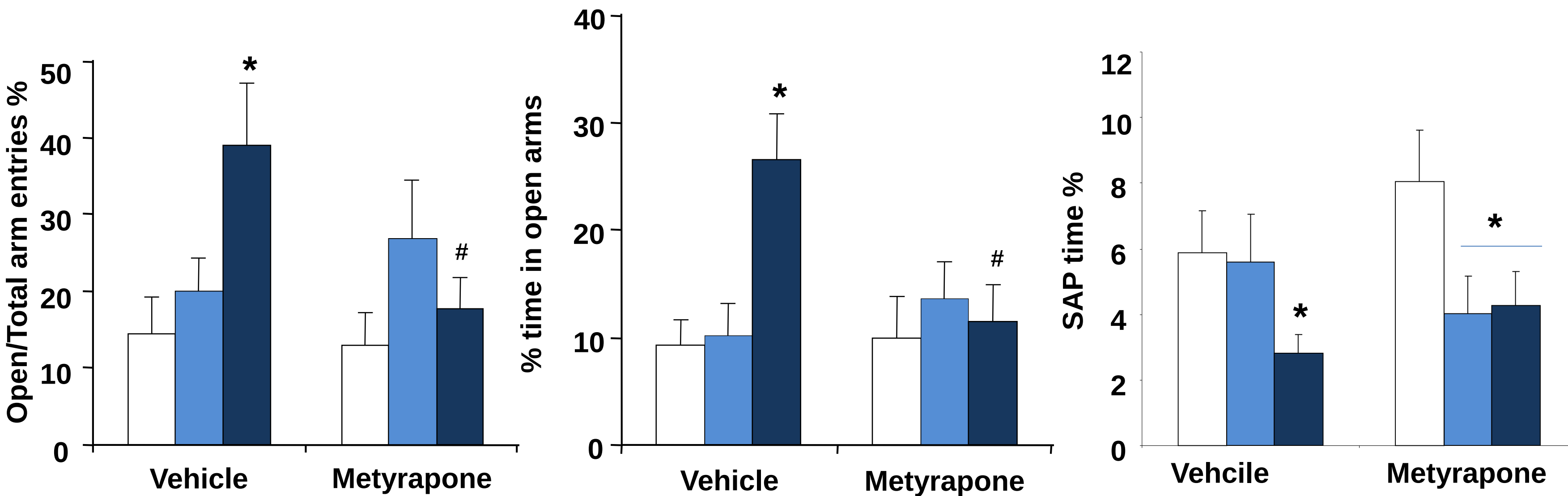


Fig.2. Enhanced 2-AG signaling significantly increased open/total arm entries and the percentage of time in open arms in the elevated plus maze test (EPM). Metyrapone administration abolished anxiolytical effects showing the CORT dependence of the mechanism.

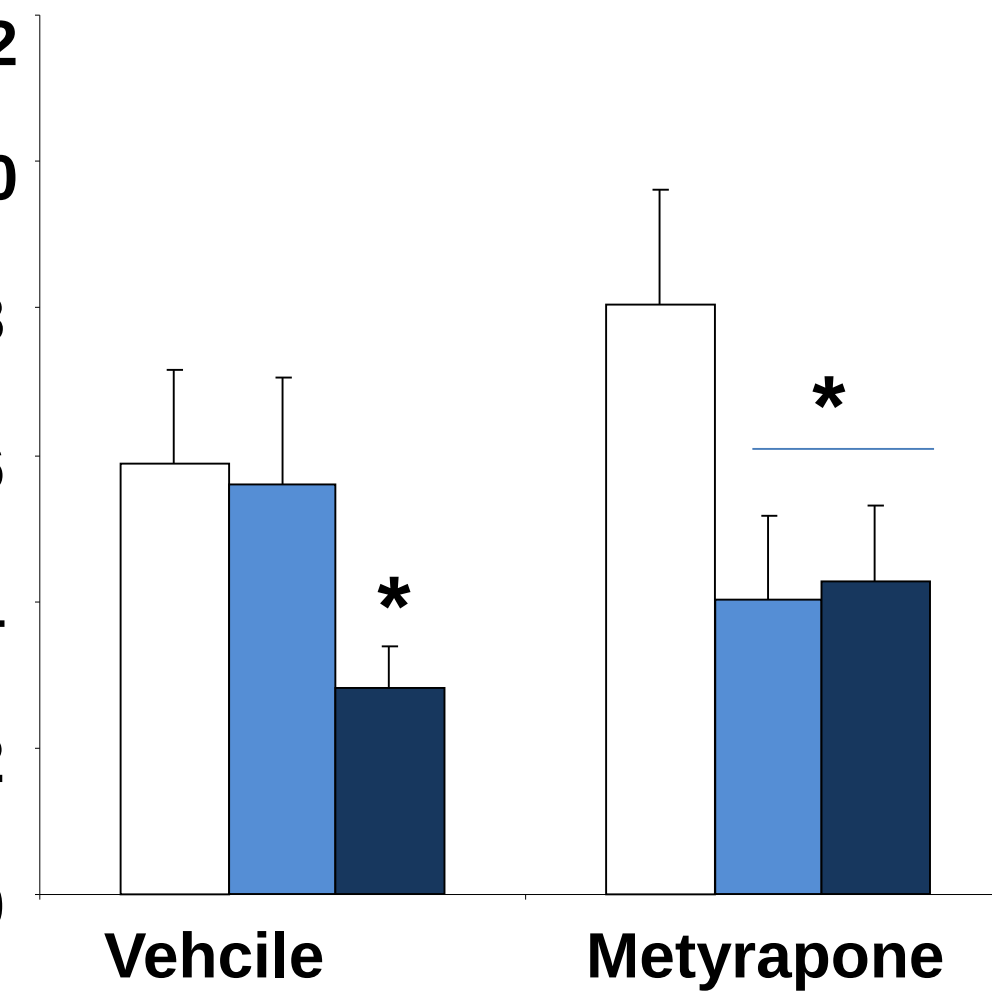


Fig.3. Enhanced 2-AG signaling significantly decreased the appearance of protected stretched attended posture (SAP) in the EPM in a CORT independent manner.

LOCOMOTION

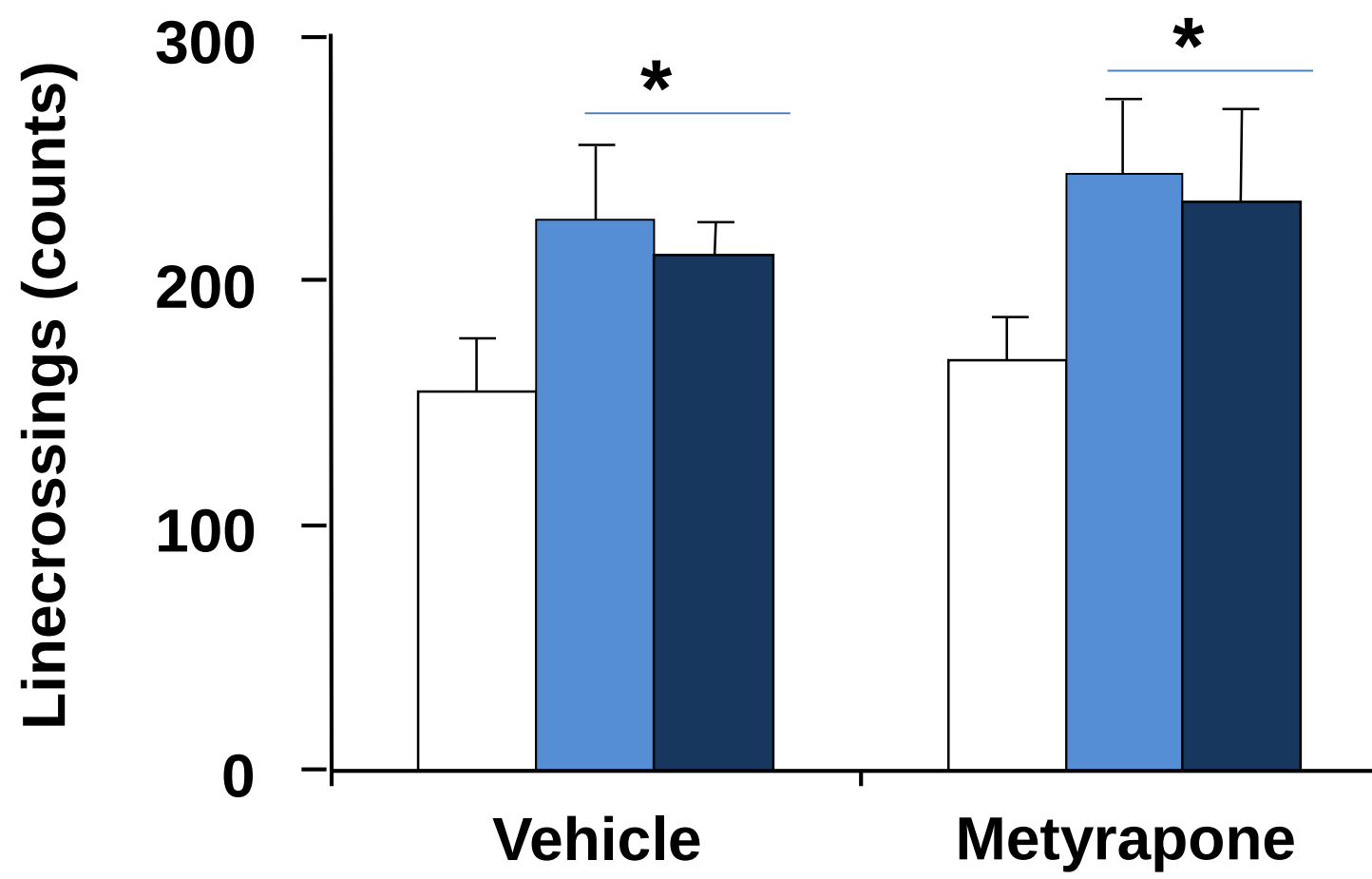


Fig.4. Enhanced 2-AG signaling significantly increased locomotion in the open field test (OF) in a CORT independent manner.

Conclusion

- Inhibition of MAGL activity enhances basal HPA-axis activity. Enhanced HPA-activity results in anxiolytic effects in a non-social challenge.
- In social challenges 2-AG signaling seems to be a strong negative modulator of aggressive behavior. This modulating effect is independent of 2-AG-induced enhanced HPA-axis activity.
- These results suggest, that the impact of 2-AG signaling on responses to social and non-social challenges is different: in the former, 2-AG alters the response *via* an intrinsic mechanism, while it alters response to the latter *via* enhanced HPA-axis activity as a secondary effect.
- Inhibition of MAGL activity enhances basal HPA-axis activity. Enhanced HPA-activity results in anxiolytic effects in a non-social challenge.
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Methods

Subjects: Adult, male CD1 mice weighing 25-40g. All experiments were carried out in the early hours of the light phase with sample sizes of 6-10 per group.

Drugs: The MAGL inhibitor JZL184 (Cayman Chemical, Ann Arbor, MI) was dissolved in 0.2 ml dimethylsulfoxide and was diluted to the final volume with saline containing 0.4% methylcellulose and administered i.p. in a volume of 10 ml/kg body weight. The corticosterone synthesis inhibitor metyrapone (Sigma Aldrich, Saint Louis, MO) was dissolved in saline containing 5% Tween 80 and administered in doses i.p. in a volume of 5 ml/kg body weight.

Behavioral testing: Behavior of the animals during the tests was recorded by a digital video camera and later analyzed by a computer based event recorder software H77 (Jozsef Haller, Institute of Experimental Medicine, Budapest, Hungary). **Forced swimming:** mice were placed in a glass cylinder (40 cm high, 14 cm diameter) filled with 35 °C degrees temperature water for 8 min. **Elevated plus-maze (EPM):** mice were placed on a black aluminum apparatus, elevated 80 cm high from the ground consisting two open (50x20cm) and two closed arms (50x20cm with 30cm high wall) for 5 min. **Open-field (OF):** mice were placed in a white, plastic box (50cmx50cm with 20cm high wall) without bedding for 15 min. **Resident/Intruder:** In the resident/intruder test, an intruder of smaller size was placed into the home cage of the subjects for 10 min.

Blood sampling and corticosterone measurement: For basal hormone measurements, blood was sampled by tail incision in EDTA-containing glass capillaries, while stress-induced levels were measured from blood samples collected from trunk blood to EDTA-containing plastic tubes after decapitation. Samples were centrifuged and blood plasma was separated. Plasma corticosterone was measured by radioimmunoassay.

Statistical analysis: Behavioral measures were assessed by ANOVA and post-hoc Duncan test. Data from in vivo biotelemetry recordings were analyzed with repeated measures ANOVA.

HPA-AXIS REACTIVITY

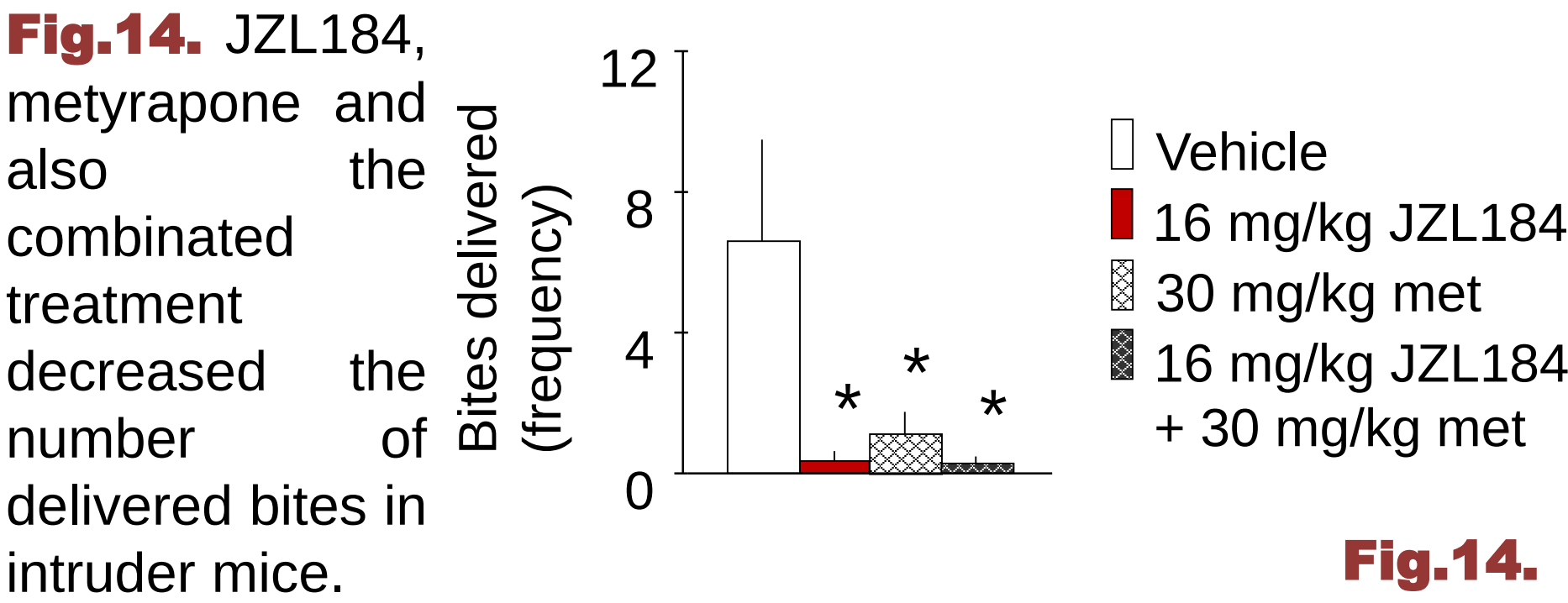


Fig.14.

CB₁R DEPENDENCE

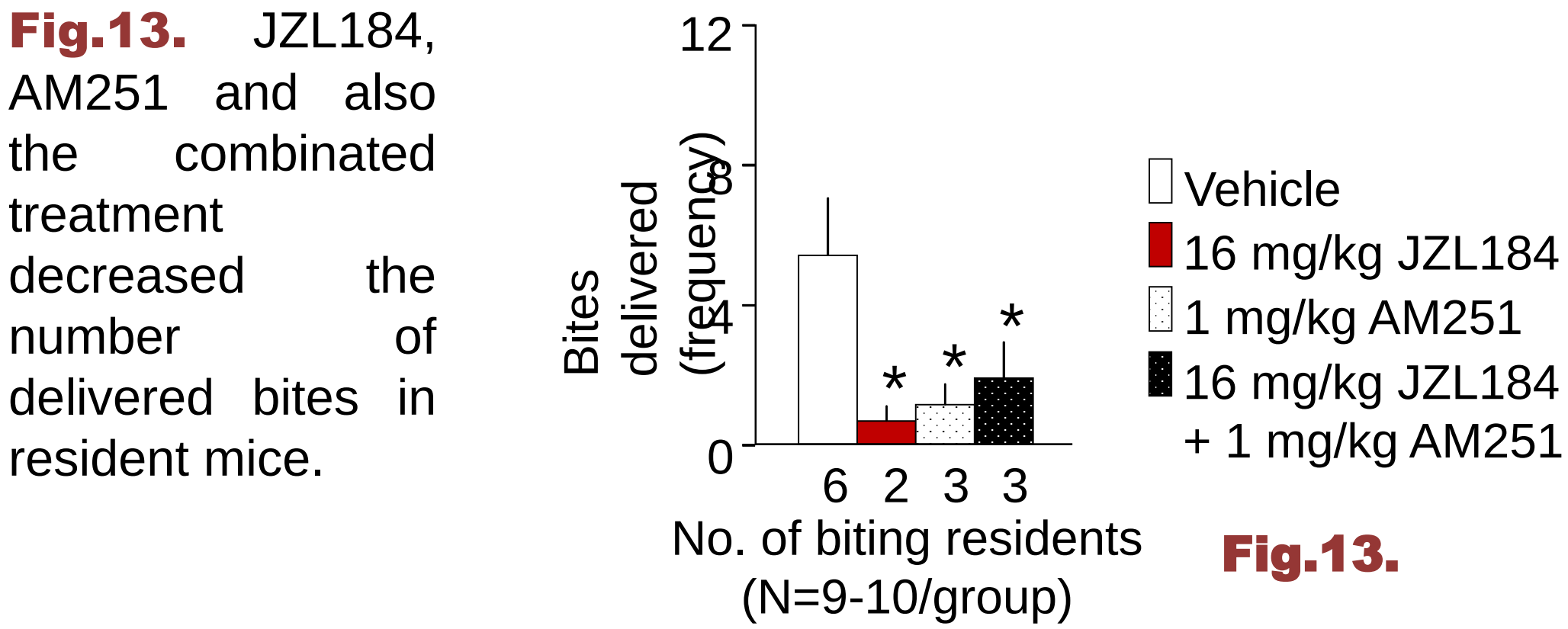


Fig.13.

Effects of MAGL blockade on behavioral and endocrine responses to social challenges

Male CD1 mice were treated with JZL184, then tested in the resident/intruder paradigm for aggression and social behavior (**Fig.5-7.,9-11.**). After testing, trunk blood was collected for stress-induced hormone measurements (**Fig.8.,12.**). In two separate experiments MAGL blockade effects were tested on resident and intruder mice as well, respectively. To control for the effects of JZL184-induced increases in plasma glucocorticoids, we co-administered corticosterone synthesis inhibitor metyrapone and JZL184 to mice, and tested them in the resident/intruder test as intruders (**Fig.14.**). We also investigated the CB₁R dependence of the effects on aggression and social behavior by the co-administration of the CB₁R antagonist AM251 (**Fig.13.**).

RESIDENTS (Fig.5-8.)

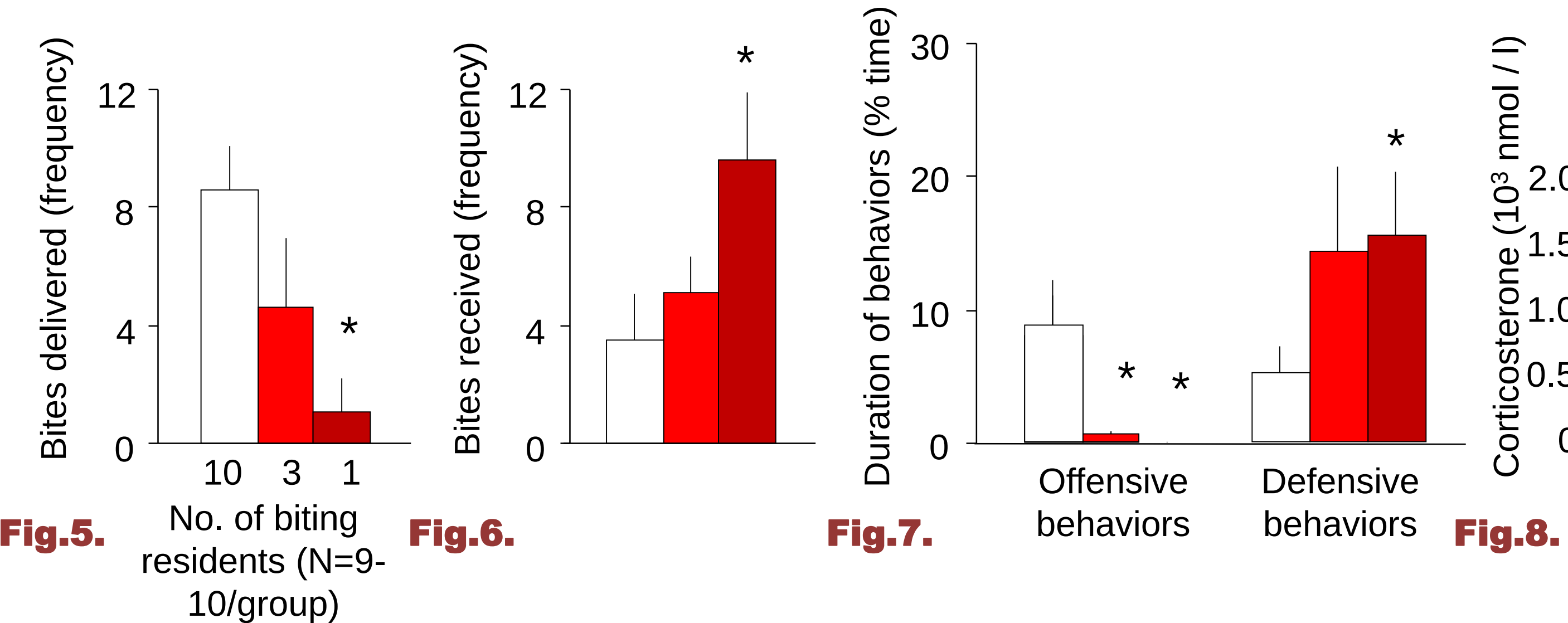


Fig.5. No. of biting residents (N=9-10/group)

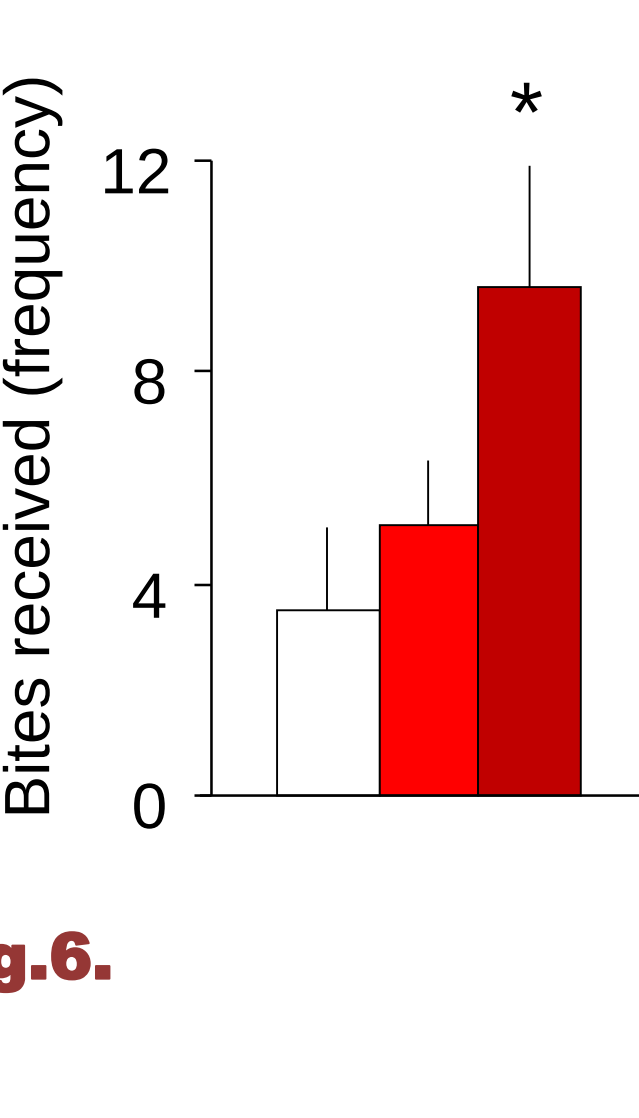


Fig.6.

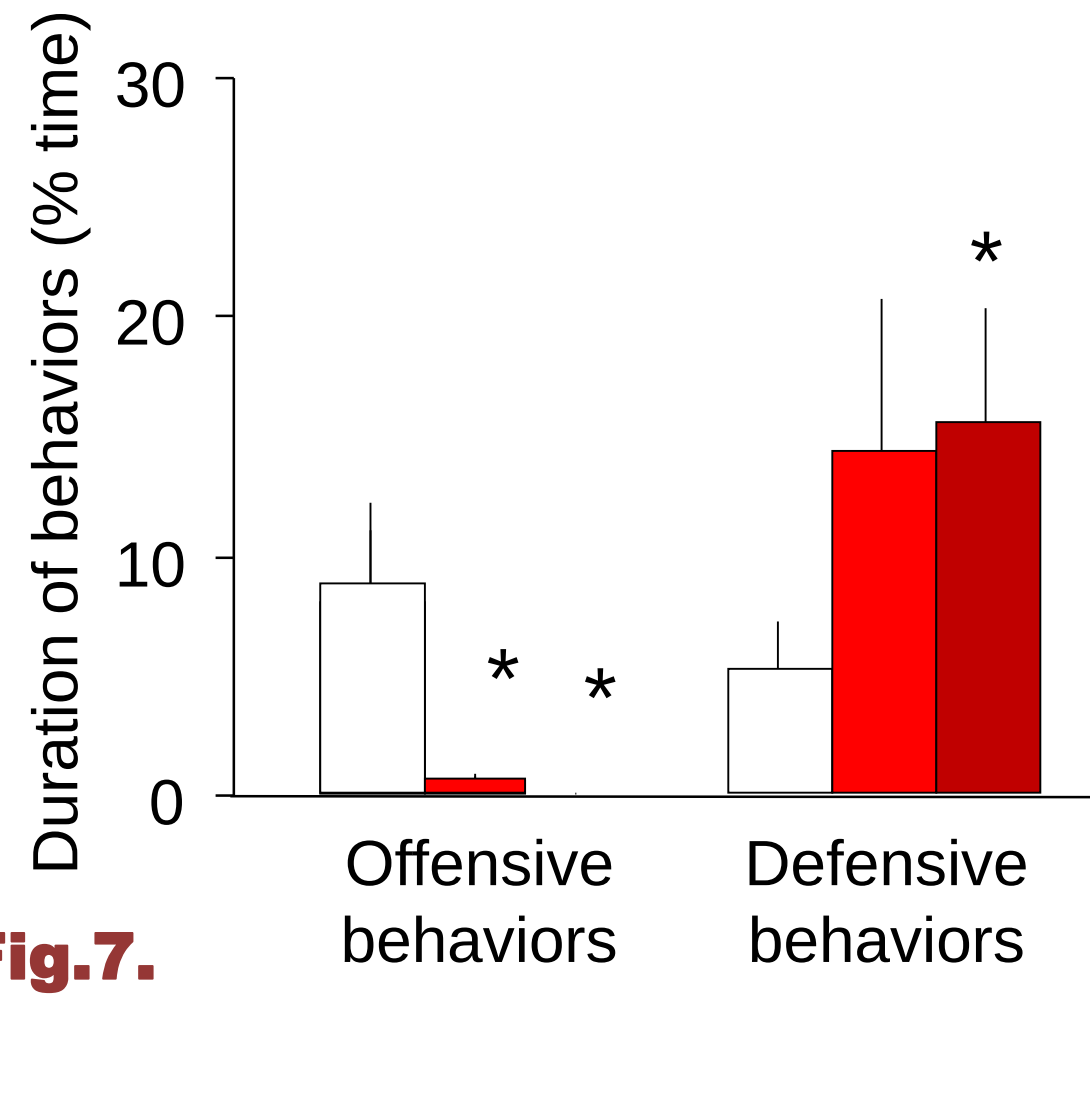


Fig.7.

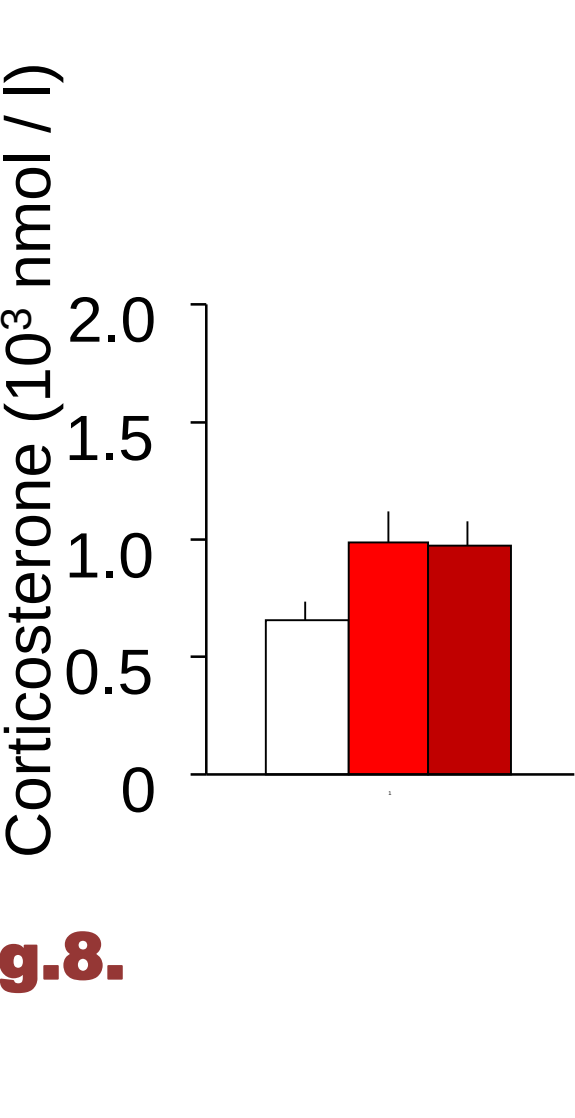


Fig.8.

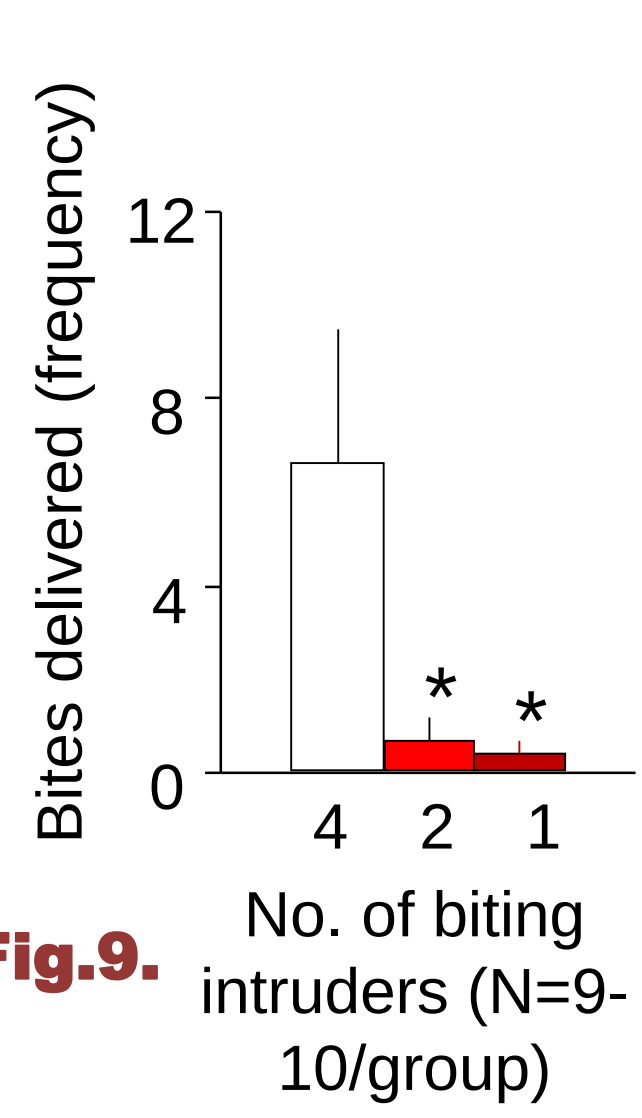


Fig.9.

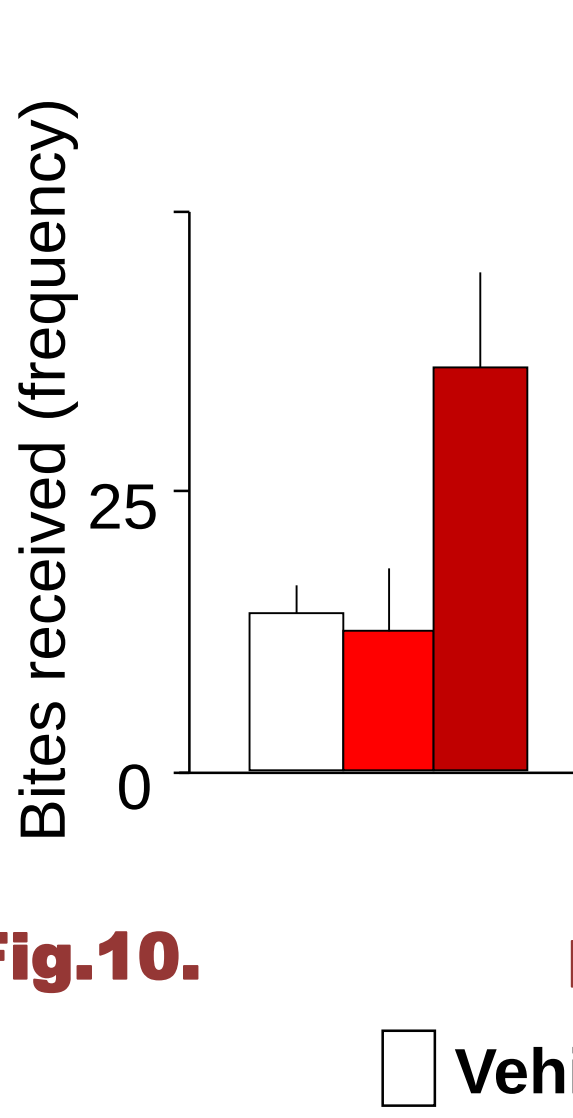


Fig.10.

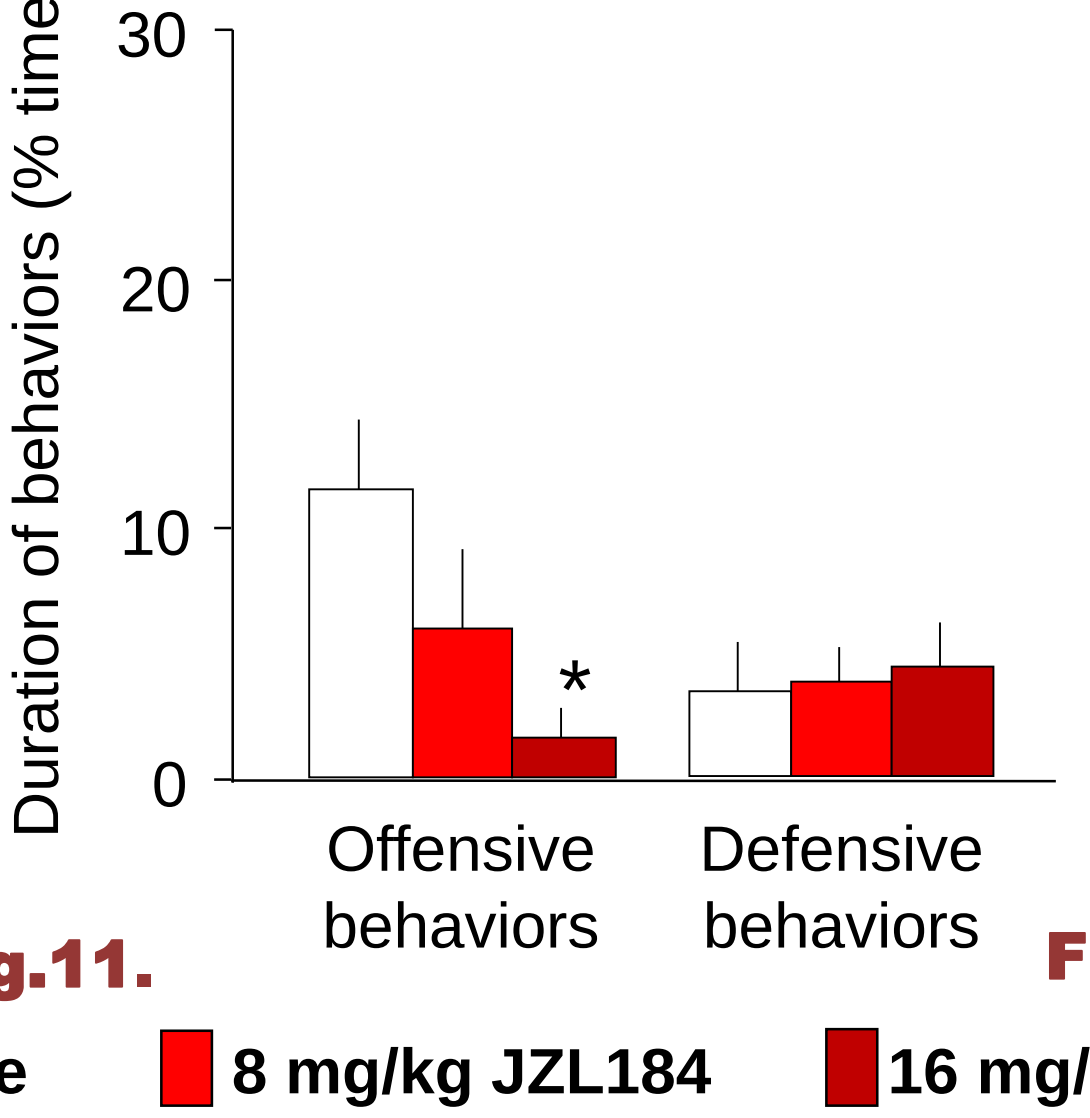


Fig.11.

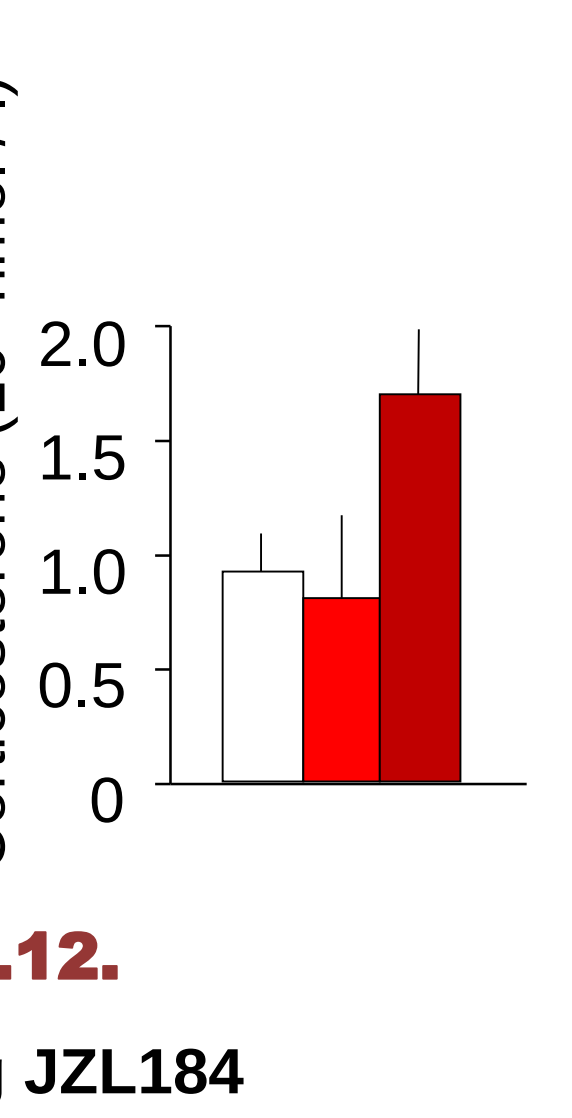


Fig.12.

JZL184 decreased the number of delivered (**Fig.5.** and **Fig.9.**), increased the number of received bites (**Fig.6.** and **Fig.10.**) and decreased offensive behavior in resident and intruder mice as well. In resident mice MAGL blockade decreased the duration of social behavior (**Fig.7.**), while in intruder mice it increased defensive behaviors (**Fig.11.**). Corticosterone levels were increased by JZL184 treatment during aggressive encounter in resident and intruder mice as well. (**Fig.8.** and **Fig.12.**). Mean±SE; *: difference from vehicle (p<0.05), marginally significant difference from vehicle (0.5 p<0.01).