FGIN-1-27, an agonist at translocator protein 18 kDa (TSPO), has anti-anxiety and anti-panic effects in non-mammalian models

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FGIN-1-27 is an agonist at the translocator protein 18 kDa (TSPO), a cholesterol transporter that is associated with neurosteroidogenesis. This protein has been identified as a peripheral binding site for benzodiazepines; in anamniotes, however, a second TSPO isoform that is absent in amniotes has been implicated in erythropoiesis. Functional conservation of the central benzodiazepine binding site located in GABA_A receptors has been demonstrated in anamniotes and amniotes alike; however, the same was not previously demonstrated for TSPO. FGIN-1-27 reduced anxiety-like behavior in the zebrafish light/dark preference test which are comparable to diazepam with fewer sedative effects. Similarly, FGIN-1-27 also reduced anxiety- and fear-like behaviors in the defense test battery in wall lizards, again producing fewer sedative-like effects than diazepam; the benzodiazepine was also unable to reduce fear-like behaviors in this species. These results A) underline the functional conservation of TSPO in defensive behavior in anamniotes; B) strengthen the proposal of using anamniote behavior as models in behavioral pharmacology; and C) suggest TSPO/neurosteroidogenesis as a target in treating anxiety disorders.

Keywords: translocator protein 18 kDa; fear; anxiety; zebrafish; wall lizard; benzodiazepines

1. Introduction

The translocator protein 18 kDa (TSPO, mitochondrial benzodiazepine receptor, peripheral benzodiazepine receptor) was first identified as a peripheral binding site for diazepam, but later identified as part of the mitochondrial cholesterol transport pathway that is associated with the regulation of cellular proliferation, immunomodulation, porphyrin transport and heme biosynthesis, anion transport, regulation of steroidogenesis, and apoptosis (Casellas et al. 2002). This transporter is highly expressed in steroidogenic tissues; in the central nervous system, its expression is mainly restricted to ependymal cells and glia, in which it is responsible for the local synthesis of neuroactive steroids such as allopregnanolone (Papadopoulos et al. 2006). This latter neurosteroid, in its turn, positively modulates GABA_A receptors, especially those involved in tonic inhibition (Smith et al. 2009; Maguire et al. 2012). As such, TSPO has been proposed as a pharmacological target for the treatment of neurological and psychiatric disorders associated with decreased GABAergic tone, such as anxiety disorders (Romeo et al. 1993; de Mateos-Verchere et al. 1998; Kita et al. 2004; Costa et al. 2011; Matsuda et al. 2011; Nin et al. 2011; Pinna and Rasmusson 2012; Pinna and Rasmusson 2012;
Perna et al. 2014) and epilepsy (Ugale et al. 2004), as well as for the fine control of stress responses (Gunn et al. 2011; Maguire et al. 2012; Maguire 2014). TSPO agonists have been demonstrated to produce anti-anxiety and anti-conflict effects in rodents with both systemic (Kita et al. 2004; Costa et al. 2011) and intra-hippocampal (Bitran et al. 2000) injections; these effects are blocked by \( \text{GABA}_A \) receptor antagonists and/or \( 5\alpha \)-reductase blockers, implicating neurosteroidogenesis and \( \text{GABA}_A \) receptors in these responses (Bitran et al. 2000). These effects are spared in adrenalectomized and castrated animals, suggesting that they are not mediated by peripheral steroidogenesis, but rather by the production of neurosteroids in the brain (Romeo et al. 1993). Nonetheless, octadecaneuropeptide, a diazepam-binding inhibitor peptide which acts through both the central benzodiazepine receptor (CBR) and TSPO, produces anxiety-like behavior in both rodents (de Mateos-Verchere et al. 1998) and fish (Matsuda et al. 2011).

TSPO is highly conserved, being present in Bacteria, Archaea and Eukarya domains (Fan and Papadopoulos 2013). Anamniotes and invertebrates possess a single isoform, while amniotes possess two TSPO isoforms (Fan et al. 2009); interestingly, while no functional divergence is predicted to appear between tspo (found in invertebrates and basal vertebrates) and tspo1 (found in amniotes), a functional divergence was detected in TSPO2 (Fan and Papadopoulos 2013). Some of the neurobehavioral functions of this protein, on the other hand, seen to be conserved. In zebrafish, for example, benzodiazepines have been shown to affect a plethora of anxiety-like behaviors, from bottom-dwelling (Bencan et al. 2009; Egan et al. 2009) and dark preference (Maximino et al. 2010; Maximino et al. 2011) to shoal cohesion (Gebauer et al. 2011) and cocaine withdrawal-induced anxiety (López-Patiño et al. 2008). Likewise, benzodiazepines decrease tonic immobility duration and the following freezing and explosive behavior in a defensive behavior battery in the wall lizard *Tropidurus oreadicus*, and also increase exploratory behavior in the same test (Maximino et al. 2014). In the separation stress paradigm, benzodiazepines attenuate separation stress-induced distress vocalizations in chicks in the anxiety phase, but not in the depression phase (Warnick et al.
Thus, agonists at the CBR decrease fear- and anxiety-like behavior in both amniotes and anamniotes. Moreover, some evidence regarding the neurosteroidogenesis pathway in behavioral control has been suggested by the observation that allopregnanolone has an anticonvulsant effect in zebrafish (Baxendale et al. 2012), and that chronic fluoxetine treatment upregulates the expression of genes from the neurosteroidogenesis pathway in this species (Wong et al. 2013). These results suggest that some downstream effectors of neurosteroidogenesis are conserved, although it is not known whether the role of TSPO in behavioral control per se is conserved. A comparative approach could untangle this question, especially if species at the base of the amniote and anamniote clades are used. In this paper, we describe the behavioral effects of FGIN-1-27, a TSPO agonist, in zebrafish *Danio rerio* and wall lizards *Tropidurus oreadicus* and compare these responses with the effects of diazepam, an agonist at the CBR.

2. Methods

2.1. Experiment 1: Effects of FGIN-1-27 and diazepam on dark preference in zebrafish

2.1.1. Animals and husbandry

XX adult zebrafish from the *longfin* phenotype were acquired in a local aquarium shop and kept in collective tanks at the laboratory for at least 2 weeks before experiments started. Conditions in the maintenance tank were kept stable, as per recommendations in Lawrence (2007). While Brazilian legislation and current guidelines do not regulate fish use in laboratory research, recommendations in Piato and Rosemberg (2014) were followed to ensure ethical principles in animal care and throughout experiments.

2.1.2. Drug administration

Diazepam was dissolved in 40% propylene glycol, 10% ethyl alcohol, 5% sodium benzoate, and 1.5% benzyl alcohol (Maximino et al. 2010). FGIN-1-27 was dissolved in 1% DMSO to which one
or two drops of Tween 80 was added before sonication into a fine suspension (Auta et al. 1993).

Drugs were diluted to their final concentrations and injected i.p. in a volume of 1 µl/0.1 g b.w. (Kinkel et al. 2010).

2.1.3. Scototaxis assay

The light/dark preference (scototaxis) assay was performed as described in Maximino et al. (2013). Briefly, 30 min after injection animals were transferred individually to the central compartment of a black and white tank (15 cm X 10 cm X 45 cm h X d X l) for a 3-min. acclimation period, after which the doors which delimit this compartment were removed and the animal was allowed to freely explore the apparatus for 15 min. While the whole experimental tank was illuminated from above by an homogeneous light source, due to the reflectivity of the apparatus walls and floor average illumination (measured just above the water line) above the black compartment was 225 ± 64.2 (mean ± S.D.) lux, while in the white compartment it was 307 ± 96.7 lux. The following variables were recorded:

- **time on the white compartment:** the time spent in the white half of the tank (percentage of the trial);
- **squares crossed:** the number of 10 cm² squares crossed by the animal in the white compartment; latency to white: the time to first entry in the white compartment (s);
- **erratic swimming:** the number of “erratic swimming” events, defined as a zig-zag, fast, unpredictable course of swimming of short duration;
- **freezing:** the proportional duration of freezing events (in % of time in the white compartment), defined as complete cessation of movements with the exception of eye and operculae movements;
- **thigmotaxis:** the proportional duration of thigmotaxis events (in % of time in the white compartment), defined as swimming in a distance of 2 cm or less from the white compartment’s
walls;

risk assessment: the number of “risk assessment” events, defined as a fast (<1 s) entry in the white compartment followed by re-entry in the black compartment, or as a partial entry in the white compartment (i.e., the pectoral fin does not cross the midline);

Video records of the experiments were manually registered by two observers blind to treatment (inter-observer reliability > 0.85) using X-Plo-Rat 2005 (http://scotty.ffclrp.usp.br).

2.2. Experiment 2: Effects of FGIN-1-27 and diazepam on the defense test battery in Tropidurus oreadicus

2.2.1. Animals and husbandry

120 adult wall lizards (Tropidurus oreadicus) of either sex, ranging from 61-96 mm in rostro-cloacal length, were captured in Marabá, PA, Brazil, between February and March. The animals were inspected for mites, which were removed with forceps before treatment with de-miting solution as described by the manufacturer (Reptile Relief, Natural Chemistry, Norwalk, USA). All of the lizards were treated with 50 mg/kg fenbendazole, p.o., and then housed according to recommendations for anoline lizards (Sanger et al. 2008) for at least 2 weeks before the experiments began. Animals were housed in groups of four in standard laboratory rat cages (42 cm length x 27.5 cm width x 21 cm height) with mango tree sticks collected from the outdoors to provide perches. Before using the sticks, they were sterilized for 15 min in an autoclave. To prevent escape, screen meshes were inserted in the cage tops. The bottoms of the cages were covered with synthetic cage carpet (Repti Cage Carpet CC-10, Zoo Med, Costa Mesa, USA) placed above a heater plate (Repti Therm U.T.H. Under Tank RH-6, Zoo Med, Costa Mesa, USA) that kept the temperature above the carpet at an average of 28°C. The cages were misted with water twice daily, thus raising the humidity within each cage to approximately 85% (Sanger et al. 2008). The animals had ad libitum access to drinking water. The animals were fed three times weekly with commercial ration (Shrimp mix, Nutral, Monte
Mor, Brazil) and once per week with captured crickets.

2.2.2. Drug administration

FGIN-1-27 and diazepam were prepared as in Experiment 1 and injected intraperitoneally with a volume of 0.5 ml of either vehicle or drug 30 min before behavioral tests.

2.2.3. Defense test battery

The defense test battery was applied as described in Maximino et al. (2014). Briefly, tonic immobility was induced by placing the animal on its back in the center of a 10 cm diameter circular open field and applying pressure to the thorax and pelvis while restraining the limbs. When the lizard ceased struggling, it was slowly released, and the time taken for it to resume an upright posture was recorded (Hennig 1979). After the animal spontaneously ceased tonic immobility, the following behavioral endpoints were recorded after each of these manipulations:

- **freezing**: the lack of limb, neck, or tongue movements for more than 5 s in an upright position;
- **circling**: a high-velocity escape attempt with a latency of less than 10 s after release, usually leading to circling around the edges of the apparatus, and quantified as the number of complete circles made near the walls;
- **tongue-flicking**: repeatedly licking the air with the tongue;

- **ventilatory frequency**: the average number of inspiratory responses per minute;
- **total locomotion**: the number of 2 cm² squares crossed by normal locomotor responses (i.e., not concomitant to circling); can be superimposed to tongue-flicking.

These behavioral endpoints were manually recorded using EthoLog 2.2 (Ottoni 2000), and the frequencies and duration were calculated.
2.3. Statistical analysis

The data were analyzed using two-way ANOVAs, with treatment and dose as between-subjects factors, followed by Tukey's HSD whenever appropriate; planned comparisons were between different doses of a given drug and its vehicle and between same doses of both drugs. All statistical analyses were made using R 3.1.3. Data are presented graphically as means ± S.E.M.

3. Results

3.1. Experiment 1

Main effects of drug ($F_{1,107} = 91.877$, $p < 0.0001$) and dose ($F_{5,107} = 36.464$, $p < 0.0001$), as well as an interaction effect ($F_{5,107} = 4.924$, $p = 0.000428$) were observed on time on white (Figure 1A); post-hoc tests uncovered differences between all doses except the highest in relation to controls in diazepam-treated animals ($p < 0.001$) and between all doses in relation to controls in FGIN-1-27-treated animals ($p < 0.001$). Finally, differences were also observed between FGIN-1-27- and diazepam-treated animals at doses of 0.56 mg/kg ($p = 0.0012$), 1.2 mg/kg ($p < 0.0001$), and 2.4 mg/kg ($p = 0.0002$).

Main effects of drug ($F_{1,107} = 17.9$, $p < 0.0001$) and dose ($F_{5,107} = 211.6$, $p < 0.0001$), as well as an interaction effect ($F_{5,107} = 20.0$, $p < 0.0001$), were found for risk assessment (Figure 1B). Post-hoc tests uncovered differences between vehicle-treated and diazepam-treated animals at all doses ($p < 0.0001$), and between vehicle-treated and FGIN-1-27-treated animals at all doses ($p < 0.0001$). Moreover, differences between diazepam- and FGIN-1-27-treated animals were observed at 1.1 mg/kg ($p = 0.012$) and 2.3 mg/kg ($p < 0.0001$).

A main effect of dose ($F_{5,107} = 111.547$, $p < 0.0001$), but not drug ($F_{1,107} = 0.643$), was found for thigmotaxis (Figure 1C); an interaction effect was also found ($F_{5,107} = 10.536$, $p < 0.0001$). Post-hoc tests unveiled differences between vehicle-treated and diazepam-treated animals at doses above 0.28 mg/kg ($p < 0.01$), as well as between all FGIN-1-27 doses and vehicle-treated animals ($p <$
Differences were also observed between diazepam- and FGIN-1-27-treated zebrafish at doses of 0.14 mg/kg (p = 0.0065) and 1.1 mg/kg (p = 0.044).

Main effects of drug ($F_{1,107} = 89.134, p < 0.0001$) and dose ($F_{5,107} = 47.418, p < 0.0001$), as well as an interaction effect ($F_{5,107} = 6.921, p < 0.0001$), were found for freezing (Figure 1D). Post-hoc tests uncovered differences between vehicle-treated and diazepam-treated animals at all doses ($p < 0.001$), and between FGIN-1-27-treated and vehicle-treated animals at 0.28 mg/kg and higher doses ($p < 0.001$). Differences were also found between diazepam- and FGIN-1-27-treated zebrafish at doses of 0.14 mg/kg ($p = 0.03$), 1.1 mg/kg ($p < 0.0001$), and 2.3 mg/kg ($p < 0.0001$).

Main effects of drug ($F_{1,107} = 14.46, p < 0.0001$) and dose ($F_{5,107} = 42.67, p < 0.0001$), as well as an interaction effect ($F_{5,107} = 26.31, p < 0.0001$), were found for erratic swimming (Figure 1E). Post-hoc tests uncovered no statistically significant differences between vehicle-treated and diazepam-treated animals at any dose ($p > 0.05$), while FGIN-1-27-treated animals were significantly different between vehicle-treated animals at doses of 0.28 mg/kg ($p = 0.0001$), 1.1 mg/kg ($p < 0.001$) and 2.3 mg/kg ($p < 0.0001$). Finally, differences were observed between FGIN-1-27- and diazepam-treated animals at 0.28 mg/kg ($p = 0.021$), 1.1 mg/kg ($p = 0.05$), and 2.3 mg/kg ($p < 0.0001$).

Finally, main effects of drug ($F_{1,107} = 9.297, p = 0.00289$) and dose ($F_{5,107} = 12.298, p < 0.0001$), as well as an interaction effect ($F_{5,107} = 4.054, p = 0.00208$), was found for number of entries on the white compartment (Figure 1F). Post-hoc tests uncovered differences between vehicle-treated and diazepam-treated zebrafish at doses of 0.28 mg/kg and 0.57 mg/kg ($p < 0.01$) and vehicle-treated and FGIN-1-27-treated zebrafish at the highest dose ($p = 0.003$); no differences were found between diazepam- and FGIN-1-27-treated animals, however.

### 3.2. Experiment 2

Main effects of drug ($F_{1,108} = 248.5, p < 0.0001$) and dose ($F_{1,108} = 93.39, p < 0.0001$), as well as an interaction effect ($F_{1,108} = 60.09, p < 0.0001$), were found for TI duration (Figure 2A). Post-hoc tests
found statistically significant differences between vehicle-treated and diazepam-treated lizards at
doses of 0.28-1.1 mg/kg (p < 0.05) and vehicle-treated and FGIN-1-27-treated lizards at all doses
except the higher (p < 0.0001). Differences between FGIN-1-27- and diazepam-treated lizards were
observed at all doses except the higher (p < 0.0001).
Main effects of drug (F₁,₁₀₈ = 771.29, p < 0.0001) and dose (F₅,₁₀₈ = 59.58, p < 0.0001), as well as an
interaction effect (F₅,₁₀₈ = 66.98, p < 0.0001), were found for circling behavior (Figure 2B). Vehicle-
treated animals differed from diazepam-treated animals at 0.14 mg/kg (p = 0.019), 0.57 mg/kg (p <
0.0001), and 1.1 mg/kg (p = 0.003); vehicle-treated lizards differed from FGIN-1-27-treated ani-
mals at all doses (p < 0.0001). Diazepam- and FGIN-1-27-treated animals differed at all doses (p <
0.0001).
Main effects of drug (F₁,₁₀₈ = 291.58, p < 0.0001) and dose (F₅,₁₀₈ = 45.09, p < 0.0001), as well as an
interaction effect (F₅,₁₀₈ = 14.63, p < 0.0001), were found for freezing (Figure 2C). Post-hoc tests
identified significant differences between vehicle- and diazepam-treated animals at 1.1 mg/kg (p <
0.0001) and between vehicle- and FGIN-1-27-treated animals at all doses (p < 0.0001). Differences
between diazepam- and FGIN-1-27-treated lizards were also found for all doses (p < 0.0001).
A drug main effect (F₁,₁₀₈ = 1482.4, p < 0.0001) and a dose main effect (F₅,₁₀₈ = 110.18, p < 0.0001),
as well as an interaction effect (F₅,₁₀₈ = 86.33, p < 0.0001), were observed for ventilatory frequency
(Figure 2D). Post-hoc tests uncovered differences between vehicle-treated and diazepam-treated an-
imals at 1.1 mg/kg (p < 0.0001), and between vehicle-treated and FGIN-1-27-treated animals at all
doses (p < 0.0001). Finally, statistically significant differences were found between diazepam- and
FGIN-1-27-treated animals at all doses (p < 0.0001).
Main effects of drug (F₁,₁₀₈ = 38.4, p < 0.0001) and dose (F₅,₁₀₈ = 437.2, p < 0.0001), as well as in-
teraction effect (F₅,₁₀₈ = 3735, p < 0.0001), were found for tongue-flicking (Figure 2E). Post-hoc
tests revealed significant differences between vehicle- and diazepam-treated lizards at doses of 1.1
and 2.3 mg/kg (p < 0.0001), and between vehicle- and FGIN-1-27-treated lizards at all doses except
the highest (p < 0.0001). Moreover, statistically significant differences between diazepam- and 
FGIN-1-27-treated lizards at all doses (p < 0.0001).

Main effects of both drug (F$_{1, 108} = 87.41$, p < 0.0001) and dose (F$_{5, 108} = 36.46$, p < 0.0001), as well 
as an interaction effect (F$_{5, 108} = 56.92$, p < 0.0001), were found for total locomotion (Figure 2F).

Significant differences were found between vehicle- and diazepam-treated lizards at 0.57 and 2.3 
mg/kg (p < 0.0003) and vehicle- and FGIN-1-27-treated lizards at 1.1 and 2.3 mg/kg (p < 0.002).

Finally, statistically significant differences were also found between diazepam- and FGIN-1-27-
treated animals at 2.3 mg/kg (p < 0.0001).

4. Discussion

In the present work we demonstrated that the TSPO agonist FGIN-1-27 produced a dose-dependent 
decrease in defensive behavior in both wall lizards and zebrafish. Specifically, in wall lizards FGIN- 
1-27 decreased tonic immobility in the intermediate dose range (0.14-1.1 mg/kg); decreased post-TI 
ventilatory frequency, freezing and circling at all doses; and increased exploratory behavior 
tongue-flicking and decreased thigmotaxis at 0.14-1.1 mg/kg). Similarly, in zebrafish FGIN-1-27 
decreased scototaxis, thigmotaxis, freezing and risk assessment at all doses, increasing erratic 
swimming at 0.14 and 0.28 mg/kg. The highest dose (2.3 mg/kg) decreased locomotion in both 
Species, suggesting a sedative effect. Moreover, diazepam, a CBR and TSPO agonist, decreased TI 
duration at the smaller doses and increased it at high doses, increased tongue-flicking and decreased 
thigmotaxis at doses above 0.28 mg/kg; no effects were observed in post-TI behavior. In zebrafish, 
diazepam produced an inverted-U effect on scototaxis and entries on white, monotonically decreasing 
freezing, thigmotaxis and risk assessment.

In the lizard defense test battery, induction of TI produces a stereotypical behavioral pattern in 
which is followed by either freezing or “explosive” circling behavior; after that, the animal emits 
exploratory behavior, marked by thigmotaxis (“wall-hugging”) and tongue-flicking (Maximino et
This sequence resembles defensive behavior at increasing predatory imminence continua (Fanselow and Lester 1988) – that is, defensive behavior in this test shifts from panic-like, circa-strike behavior towards sustained risk assessment (tongue-flicking, thigmotaxis). Moreover, in a previous experiment panicolytic drugs (alprazolam, imipramine) decreased TI duration, freezing and circling, while diazepam (0.5 mg/kg) increased tongue-flicking and decreased thigmotaxis (Maximino et al. 2014). In the present experiment, FGIN-1-27 produced a more wide range of effects than diazepam: the TSPO agonist affected both exploratory behavior (consistent with an anxiolytic-like effect), post-TI behavior (consistent with a panicolytic effect) and TI duration, which could also represent a panicolytic-like effect; diazepam, on the other hand, only affected exploratory behavior and had a hormetic (inverted-U) profile on TI duration.

A complementary profile was observed in the zebrafish scototaxis assay. Benzodiazepines have been shown to produce an hormetic profile in several behavioral tests in this species (Bencan et al. 2009; Cachat et al. 2010; Sackerman et al. 2010; Vada et al. 2015). Previous experiments also demonstrated that diazepam was anxiolytic at 1.25 mg/kg, but not 2.5 mg/kg, in the scototaxis assay (Maximino et al., 2011); in addition to this observation, the present work also demonstrated effects of diazepam on freezing, thigmotaxis and risk assessment. Importantly, FGIN-1-27 treatment also produced anxiolytic-like effects, with locomotor-impairing effects at the highest dose.

Little is known about the role of TSPO in behavioral control in anamniotes. In goldfish Carassius auratus, octadecaneuropeptide increased the latency to enter a white compartment in a light/dark box, suggesting an anxiogenic-like effect (Matsuda et al. 2011). While octadecaneuropeptide is an endozepine which acts as an agonist at TSPO (Papadopoulos et al. 1991), it also acts as an antagonist at the CBR (Ferrero et al. 1986); consistently with the hypothesis that the anxiogenic-like effect of octadecaneuropeptide is mediated by the CBR, flumazenil, but not a metabotropic endozepine receptor antagonist, blocked the effects on goldfish scototaxis (Matsuda et al. 2011).

Overall, the present results suggest that drugs targeting the CBR and MBR exert anti-anxiety effects.
in anamniotes, while drugs acting at the MBR also exert anti-panic effects. It is plausible that some
of the effects of diazepam were mediated by the MBR; further experiments are needed to untangle
the precise mechanisms. Thus, while the present results suggest a functional conservation of TSPO
that is concomitant to gene duplication, it is not known whether FGIN-1-27 (or even diazepam) pro-
duced its behavioral effects in lizards and zebrafish by acting on the (conserved) tspo1 or whether
some effects are also mediated by tspo. Further experiments will clarify the issue. The present re-
results also add to the mounting evidence that TSPO/MBR ligands could be used to treat fear disor-
ders, including panic disorder, in human populations; the degree of conservation suggest that anam-
niotes could be used as experimental models to study anti-panic drugs.

References


Hennig CW (1979) The effects of physical environment, time in captivity, and defensive distance on tonic immobility, freezing, and flight behaviors in Anolis carolinensis. Anim Learn Behav 7:106–110.


Figure captions

**Figure 1** – Effects of FGIN-1-27 (filled circles) and diazepam (empty circles) on (A) scototaxis, (B) risk assessment, (C) thigmotaxis, (D) freezing, (E) erratic swimming and (F) total locomotion in the light/dark test in zebrafish (*Danio rerio*). Error bars represent to standard errors.
Figure 2 – Effects of FGIN-1-27 (filled circles) and diazepam (empty circles) on (A) tonic immobility, (B) circling responses, (C) freezing, (D) ventilatory frequency, (E) tongue-flicking and (F) total locomotion on wall lizards *Tropidurus oreadicus*. Error bars represent standard errors.