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Sigma-1 receptor knockout mice display a depressive-like phenotype

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Abstract

Activation of sigma-1 receptors (Sig-1R) reportedly has antidepressant-like action. Limited data suggest that Sig-1Rs also modulate anxiety-related behaviors. The present experiments measured depressive-like, anxiety-like and motor behavior in Sig-1R knockout mice and their wildtype littermates. Sig-1R knockout mutants showed increased immobility in the forced swimming test, a depressive-like phenotype, but normal anxiety-like behavior in the elevated plus-maze and light/dark box tests and normal locomotor activity. The results further suggest that Sig-1Rs inversely modulate depressive-like behavior.

Sigma receptors (SigRs) are non-opioid, non-phencyclidine, intracellular receptors that modulate multiple signal transduction and neurotransmitter systems. Two different SigR subtypes are known, Sig-1R and Sig-2R, which differ in their binding profile and molecular weight [17,20,34]. The Sig-1R gene [33,37,44] encodes a 29 kDa polypeptide containing one or two putative transmembrane domains. Sig-1Rs are widely expressed in rat brain, especially within limbic systems and brainstem motor structures. The highest levels of Sig-1R immunostaining are observed in the olfactory bulb, hypothalamus and hippocampus, with the caudate-putamen, septum, nucleus accumbens and amygdala also showing moderately concentrated, intense labeling [2,6,38]. In animal models, blockade of Sig-1Rs is known to attenuate the rewarding and toxic properties of drugs of abuse, including psychostimulants and ethanol [18,28,32,42]. Conversely, activation of Sig-1R exerts neuroprotective effects and attenuates learning and memory impairments [11,30,31].

More recently, Sig-1R systems have also been studied for their possible relation to depressive- or anxiety-related behavior. Mounting pharmacological data suggest an antidepressant-like action of selective Sig-1R agonists. Chemically unrelated Sig-1R agonists, such as (+)-pentazocine, 1,3-di-o-tolyguanidine (DTG), SA-4503, igmesine and additional novel ligands, dose-dependently reduce immobility in animal models of behavioral despair, including the

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forced swim and/or tail suspension tests; the antidepressant-like action induced by these ligands is reversed by the selective Sig-1R antagonist NE-100 [1,29,46,49]. Moreover, antidepressant-like actions of the neurosteroids dehydroepiandrosterone sulfate and pregnenolone, putative endogenous ligands for Sig-1Rs, also appear to be mediated, at least partly, by Sig-1R receptors [15,41,47]. Finally, many structurally distinct psychotropic drugs used clinically as antidepressants bind Sig-1Rs with high affinity [23,35,43,50]; in animal models, antidepressant-like actions of several such compounds, including fluovoxamine, venlafaxine and bupropion, can be abolished by pretreatment with a Sig-1R antagonist [14,16,49].

The few studies that have investigated the role of Sig-1Rs in anxiety-related behavior have obtained conflicting results. The SigR agonists (+)-SKF-10,047 and dextromethorphan, but not (+)-pentazocine or DTG, ameliorated conditioned fear stress [24,36]. A patent application asserted that disubstituted guanidines with high affinity for SigRs exert anxiolytic-like activity (http://www.wipo.int/pctdb/en/wo.jsp?WO=1990014067&IA=US1990002398&DISPLAY=DESC), a claim not yet peer-reviewed.

Recently, separate lines of Sig-1R knockout (Sig-1R KO) mice were generated by gene targeting ($Oprs1^{tm1Lmon}/Oprs1^{tm1Lmon}$) [26] and gene trapping ($Oprs1^{Gt(IRESBetageo)33Lex}/Oprs1^{Gt(IRESBetageo)33Lex}$) methods. Both knockout models were viable and fertile with negligible overt phenotype observed in cursory observations using limited sample sizes; the former model showed only a blunted hypomotility response to the SigR agonist (+)-SKF-10,047 [7,26] (http://www.informatics.jax.org/external/ko/lexicon/2691.html). Because of the suggestive pharmacological evidence suggesting a role of Sig-1Rs in depressive- or anxiety-like behaviors, the present study tested large samples of $Oprs1^{Gt(IRESBetageo)33Lex}/Oprs1^{Gt(IRESBetageo)33Lex}$ null mutant and wildtype littermate mice in selected tests of depression- and anxiety-related behaviors.

Heterozygote Oprs1 mutant (+/-) $Oprs1^{Gt(IRESBetageo)33Lex}$ embryos on a C57BL/6J × 129S/SvEv mixed background were obtained from the Mutant Mouse Resource Regional Center (MMRRC) and implanted into females C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) at The Scripps Research Institute. The first generation yielded wild-type (+/+, WT) and heterozygous mice (+/-) which were then subjected to heterozygous mating. The resulting male, adult null mutant mice (Oprs1 -/-, Sig-1R KO) and their age-matched, WT littermates were subjects in this study. Reverse transcriptase-PCR was used to assess the presence vs. absence of the native Oprs1 transcript [primers sequences:a) 5'-TCTGAGTACGTGCTGCTCTTCG-3', b) 5'-ATAAACCCTCTTGCAGTTGCATC-3', c) 5'-GAAACTGCCGTGTTCTGTTTCC-3', PCR reaction conditions: 30 cycles of 94°C (15 sec), 55°C (30 sec) and 72°C (40 sec)]. Mice (*n*=17-18/genotype), 6-8 months of age, weighing 34.26 g ± 0.84 on average, were group-housed (3-5/cage) in Macrolon shoebox cages, with free access to food and water, in a in a humidity- and temperature (22°C)-controlled vivarium on a 12-h light–dark cycle (lights off, 9:00 am). Mice were allowed to habituate to the testing environment for 1 h prior to all tests. Experiments were performed during the first half of the dark cycle. Procedures adhered to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute. A single cohort of animals was used, per the following test sequence: elevated plus-maze –light–dark transfer -forced swim test –motor activity. Tests were spaced by at least two weeks, and the order of testing was chosen such that tests involving lower stress levels (elevated plus-maze and light–dark transfer) preceded those involving higher stress levels (forced swim). Non-computerized tests were videotaped and later scored by a single rater naïve to genotype. Slight differences in sample sizes across tests reflect procedural errors, including video recording failures or animals not completing testing due for example to falling off the apparatus, frequencies of which did not differ per genotype.

The elevated plus-maze test was performed as previously described [22,27]. The plus-maze consisted of two open and two closed arms (each 30cm × 5cm, 30cm above the ground), extending horizontally at right angles from a central square (5cm × 5cm). The closed arms were enclosed by clear Plexiglas walls (30cm high), whereas the open arms only had a 0.5cm clear Plexiglas lip around their edges. Lighting on the open arms was ~2 lux. Mice were placed in the central area facing an open arm and allowed to explore for 5 min. Open and closed arm entries (all four paws in the arm) and time spent in the open arms, closed arms, and center square were scored. The % open arm time, an inverse measure of anxiety-like behavior, was calculated as [time in open arms/(total time in arms)*100] [48].

The light–dark transfer test was performed as described previously [3,4,9] with minor modifications. The test apparatus was a Plexiglas rectangular box divided into two unequal compartments by a black Plexiglas partition with a small hole at the base (7.5 × 7.5 cm). The smaller compartment (14.5 × 27 × 26.5 cm) was dark (~0 lux) and the larger compartment (28.5 × 27 × 26.5 cm) was brightly illuminated (900 lux) with a 75 W light source located above it. To assess initial emergence behavior, mice were placed in the center of the dark compartment facing away from the partition to initiate the test session. The latency to enter the light compartment, the number of transitions between light and dark compartments and the % of time spent in the light compartment during each 10-min test session were scored.

Forced swim testing was adapted from the behavioral despair test described by Porsolt [39, 40], using a larger diameter cylinder to increase sensitivity, as described previously [8,45]. Under light and within the first half of the dark cycle, mice were individually placed in two clear polypropylene cylinders (38 cm tall, 27 cm diameter; Cambro, Huntington Beach, CA) that were separated from one another by an opaque screen and which contained 23-25°C water 30 cm deep to prevent the mouse's tail from touching the cylinder bottom [12,13]. The water was changed between subjects. A subject's behavior was scored for the last 4 min of the 6-min test session [39,40] via a previously validated time-sampling method in which the presence of immobility, swimming, or climbing was rated at 5-sec intervals [12].

The motor activity apparatus consisted of 16 wire-mesh cages with disposable cardboard floors (20×25×36 cm), each equipped with two horizontal infrared photocell beams situated along the long axis of the cage, 2 cm above the floor and 16 cm from one another. Mice were individually placed into the unfamiliar apparatus during the first half of the dark cycle, and photocell interruptions were recorded automatically by a computer throughout the 10-min testing period with white noise (70 dB) present.

In the forced swim and motor behavior tests, genotype comparisons involved a two-way mixed design analysis of variance (ANOVA) on incremental data; genotype was a between-subjects factor and time a within-subject factor. Differences between the two genotypes at each time point were analyzed using Student's unpaired *t*-test. Genotype differences in the light-dark transfer and elevated plus-maze tests were analyzed using Student's unpaired *t*-test.

In the light-dark transfer test of anxiety-like behavior, no significant genotype effects were found in the % of time spent in the light compartment of the apparatus [$t(1,34) = 0.28$; n.s.] (Fig. 1a), in the latency to first enter into the light compartment [$t(1,34) = 1.02$; n.s.] (Fig. 1b), or in the total number of transitions between compartments [$t(1,34) = 0.10$; n.s.] (Fig. 1c). Similarly, in the elevated plus maze test, no significant genotype effects were found in the % of time spent on the open arms [$t(1,33) = 0.17$; n.s.] (Fig. 1d), a measure of anxiety-like behavior, or in the number of entries in the closed arms [$t(1,33) = 0.38$; n.s.] (Fig. 1e), a measure of non-specific motor activity.

In the forced swim test, Sig-1R KO mice showed significantly greater immobility [$F(1,32) = 4.39$; $p < 0.05$] (Fig. 2a) and less swimming than WT mice [$F(1,32) = 6.62$; $p < 0.05$] (Fig. 2b),

a depressive-like phenotype. Pairwise comparisons showed that the two genotypes differed significantly during minutes 5 and 6 of the test session for both measures, leading to different cumulative scores. Sig-1R KO and WT showed similar levels of climbing [$F(1,32) = 0.01$; n.s.] (Fig. 2c).

The motor activity of the two genotypes in an unfamiliar environment did not differ across the 10-min observation period [$F(1,34) = 0.24$; n.s.], as shown in Fig. 3. The duration of the observation period was chosen to match the duration of the longest behavioral test (light-dark transfer) and to encompass the time frame of forced swim testing (minutes 3-6).

The present results show that mice which lack Sig-1R due to retroviral disruption of the Oprs1 gene show a depressive-like, but not anxiogenic-like, phenotype. Sig-1R KO mice exhibited a 30-35% increase in forced swim immobility time compared to WT mice, in the context of normal levels of climbing and normal levels of general motor activity in two other novel environments, the motor activity and elevated-plus maze tests. The depressive-like phenotype of Sig-1R KO mice is reciprocally consistent with the replicated finding that numerous Sig-1R agonists, including (+)-pentazocine, DTG, SA-4503, igmesine, and novel compounds of the UMB series, as well as neurosteroids such as dehydroepiandrosterone sulfate and pregnenolone, reduce immobility time in the forced swim and tail suspension tests in a Sig-1R antagonist reversible manner [1,15,16,29,41,46,47,49]. In addition, for several clinically used antidepressants, binding with the Sig-1R is important for their antidepressant-like activity in animal models [14,16,49].

The mechanism by which Sig-1Rs may modulate antidepressant-like behavior is not yet clear, but the present and previous results raise the hypothesis that modulation of serotonergic transmission may be involved. Sig-1R KO mice showed selective reductions in swimming, and not climbing, behavior in the forced swim test. Accordingly, Detke, Lucki, Cryan and colleagues previously showed in the rat forced swim test that selective serotonin reuptake inhibitors and serotonin 1A receptor agonists selectively increase swimming, but not climbing, behavior, whereas tricyclic antidepressants and selective norepinephrine reuptake inhibitors preferentially increase climbing, but not swimming, behavior [10,12]. The generalizability of this neuropharmacological specificity to the mouse forced swim test remains uncertain. However, also supporting the hypothesis that the increased depressive-like behavior observed in Sig-1R KO mice may relate to deficits in serotonergic neuronal activity, electrophysiological studies showed that SigR agonists, such as (+)-pentazocine and DTG, increase the firing of serotonergic neurons in the dorsal raphe nucleus, leading to an enhancement of serotonergic neurotransmission [5]. Follow-up molecular studies of serotonergic function and of the responsivity of Sig-1R KO mice to antidepressant-like activity of selective serotonin vs. noradrenergic reuptake inhibitors can help evaluate this hypothesis further.

Another interpretation is that the depressive-like behavior of Sig-1R KO mice might result from a dysregulated endoplasmic reticulum (ER) stress response. Growing evidence indicates that cellular stress signaling and ER stress specifically are involved not only in the pathophysiology of neurodegenerative diseases but also in that of mood disorders (for a review see [51]). Furthermore, it has been demonstrated that mood-stabilizing drugs, such as valproate and lithium can increase the expression of ER chaperones, which have neuroprotective properties and are induced in conditions of ER stress [25]; [21]. Because Sig-1Rs are putative ligand-operated receptor chaperones that counteract ER stress, especially in response to altered calcium signaling in the ER. As a result, Sig-1Rs have been proposed to be cytoprotective [19]. It might be speculated that the depressive-like behavior in Sig-1R KO mice might arise from the inability of these mice to respond efficiently to ER stress that can occur during signal transduction, leading to impaired neuronal function.

In summary the present results with Sig-1R KO mutants implicate a role for Sig-1Rs in depressive-like behavior, findings which further validate Sig-1R agonists as potential therapeutics for depressive disorders.

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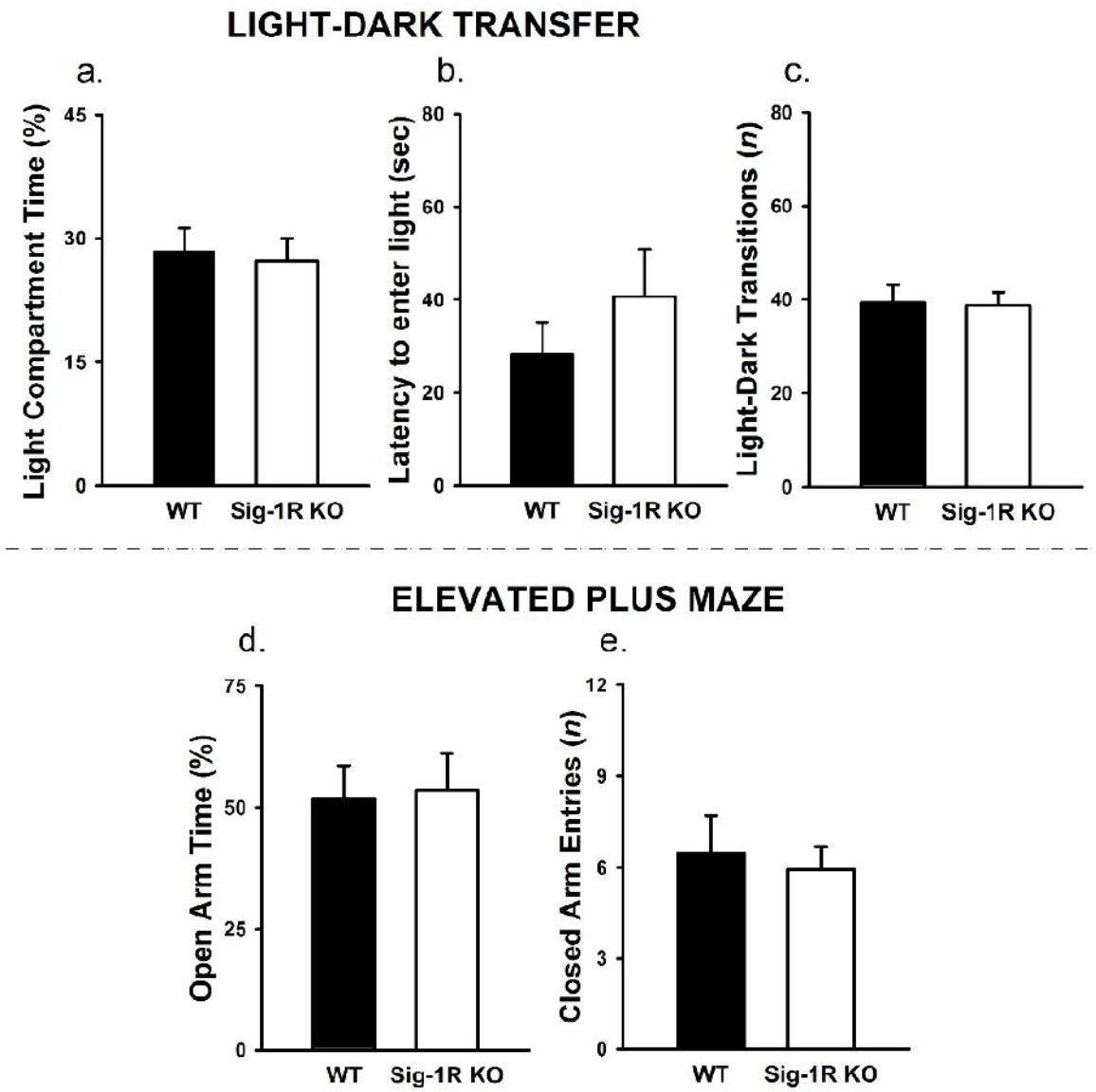

Fig. 1.

Sig-1R KO mice do not differ from WT mice in the light-dark transfer or elevated plus-maze tests of anxiety-like behavior.

Light-dark transfer: (a) Sig-1R KO mice (*n*= 18) did not differ from their WT littermates controls (*n*= 18) in the % of time spent in the light side of the light–dark box. (b) Upon initial placement in the dark side of the light–dark box, latency to first entry into the light side did not differ between Sig-1R KO and WT mice. (c) Sig-1R KO mice made the same number of transitions between sides of the light–dark box as WT mice. *Elevated plus maze:* (d) Sig-1R KO mice (*n*= 17) did not differ from their WT littermates controls (*n*= 18) in the % of time spent in the open arms of the maze, a measure of anxiety-like behavior. (e) Sig-1R KO and WT mice made the same number of entries into the closed arms of the maze, a measure of general motor activity. Data represent Mean + SEM.

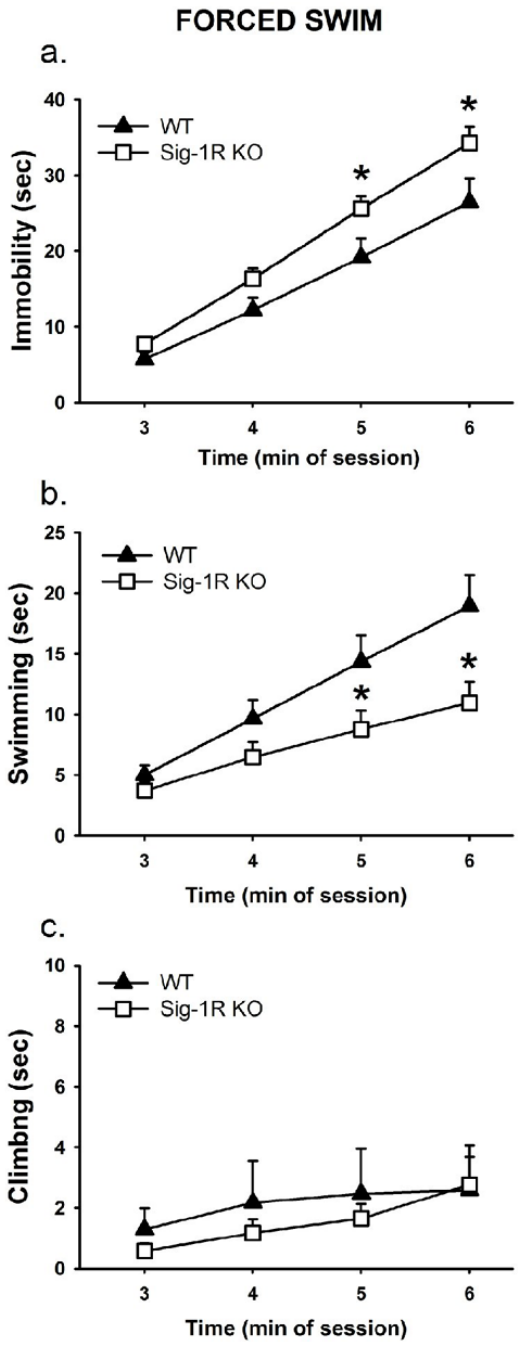

Fig. 2.

Sig-1R KO mice exhibit increased depressive-like behavior in the forced swim test. (a) Sig-1R KO mice (*n*= 17) showed more immobility behavior than WT mice. (b) Sig-1R KO mice exhibited less swimming behavior than WT mice. (c) Sig-1R KO mice did not differ in climbing behavior from WT mice. Note *y*-axis scale differences. Data represent Mean + SEM. *$p < 0.05$ (unpaired t-test).

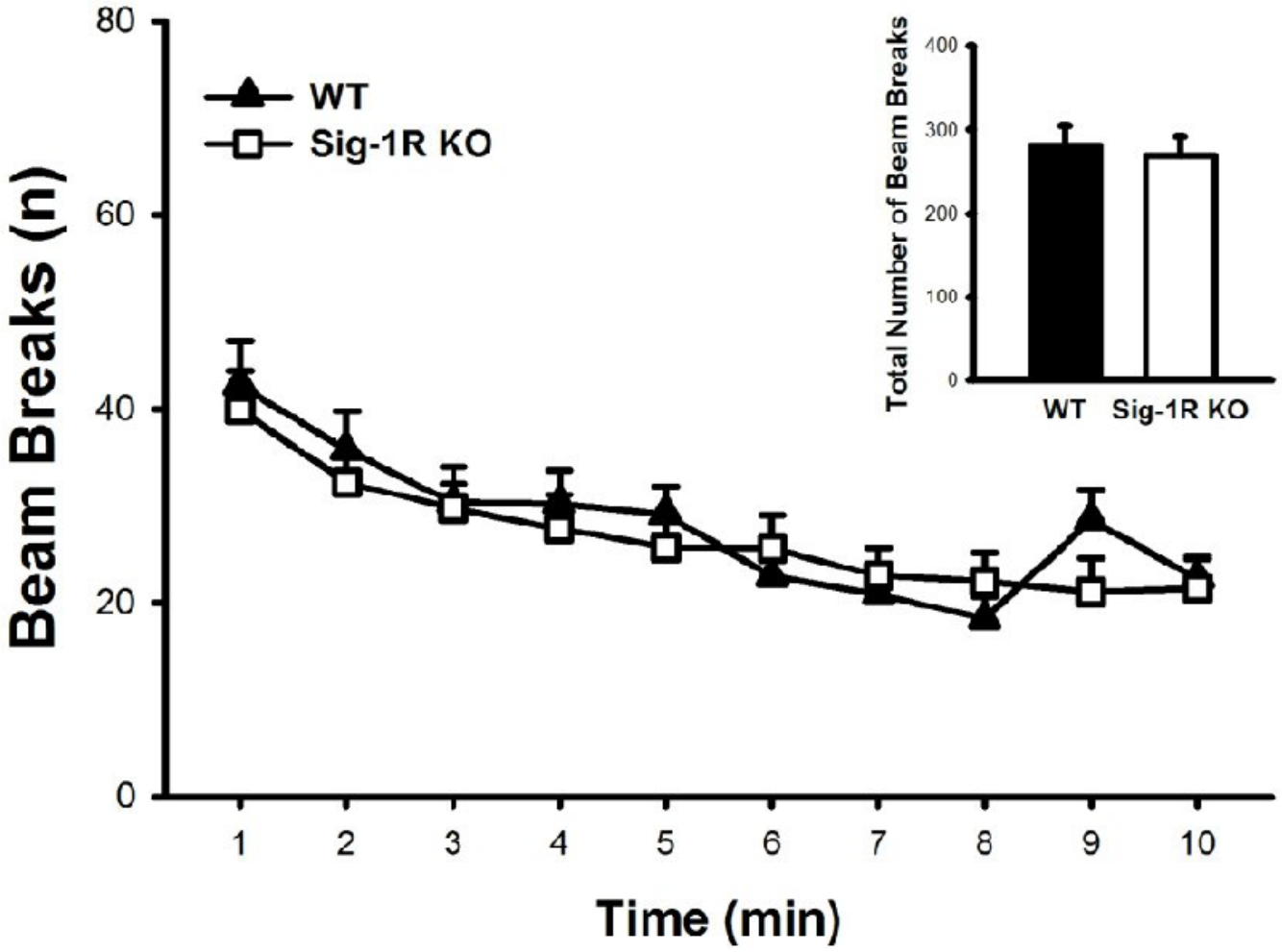

Fig. 3.

Sig-1R KO mice do not differ from WT mice in motor activity in an unfamiliar environment. Sig-1R KO mice (*n*= 18) did not differ from their WT littermates controls (*n*= 17) in the number of beam breaks across the testing period. The inset shows the cumulative number of beam breaks in the whole 10 minutes. Data represent Mean + SEM.