# Effects of (+) SKF 10,047, a sigma-1 receptor agonist, on anxiety, tested in two laboratory models in mice

José Francisco Navarro[1], David Beltrán[2] y María Cavas[1]
[1] Universidad de Málaga y [2] Universidad de La Laguna

Recently, sigma-1 receptor modulators have been considered drugs with an interesting therapeutic potential for the treatment of anxiety. However, there is no clear information in preclinical studies about the possible effects of sigma-1 ligands on anxiety in experimental animal models. Therefore, the present study examined the effects of (+)SKF 10,047 (2-8 mg/kg, ip), a sigma-1 agonist, on anxiety, tested in two classical laboratory models (social interaction test and elevated plus maze). (+)SKF 10,047 (8 mg/kg) produced a significant decrease of social investigation in the "social interaction test", whereas in the "elevated plus maze", the drug (4 and 8 mg/kg) provoked a significant reduction in the number of entries into open arms, as well as in the time spent in this area, as compared with the control group, without affecting motor activity. Overall, these findings indicate that (+)SKF 10,047 exhibits an anxiogenic-like profile in mice. It is suggested that anxiogenic effects of this sigma-1 ligand could be related to its potent ability to modulate diverse neurotransmitter systems involved in anxiety regulation.

*Efectos del (+)SKF 10,047, un agonista del receptor sigma-1, sobre la ansiedad evaluada en dos modelos de laboratorio en ratones.* Los receptores sigma-1 han sido considerados recientemente como fármacos con un interesante potencial terapéutico para el tratamiento de la ansiedad. Sin embargo, no existe información clara en estudios preclínicos con respecto a los posibles efectos de los ligandos sigma-1 sobre la ansiedad en modelos animales experimentales. Por lo tanto, en este trabajo examinamos los efectos de la administración de (+)SKF 10,047 (2-8 mg/kg, ip), un agonista sigma-1, sobre la ansiedad evaluada en dos modelos clásicos de laboratorio (test de interacción social y laberinto elevado en cruz). (+)SKF 10,047 (8 mg/kg) produjo una reducción significativa de las conductas de investigación social en el "test de interacción social", mientras que en el laberinto elevado en cruz el fármaco provocó (4 y 8 mg/kg) una disminución significativa del número de entradas y del tiempo pasado en los brazos abiertos del laberinto, en comparación con el grupo control, sin afectar la actividad motora. Estos resultados indican que el (+)SKF 10,047 muestra un perfil ansiogénico en ratones. Se sugiere que los efectos ansiogénicos de este ligando sigma-1 podrían estar relacionados con su potente capacidad para modular diferentes sistemas de neurotransmisión implicados en la regulación de la ansiedad.

Although the sigma binding site was initially described as a subtype of opiate receptors, it is now well established that sigma receptors represent an unique binding site in brain, distinct from any other known proteins. To date, two subclasses of sigma receptors have been identified, termed sigma-1 and sigma-2, differentiable by pharmacological profile, function, and molecular size (Bowen, 2000; Beltrán, Cavas, & Navarro, 2004a). However, this classification remains unsatisfactory and there is controversy over the existence of a third subtype of sigma receptors (Kulkarni & Dhir, 2009).

The anatomical distribution of sigma-1 in the central nervous system has been well characterized. Thus, binding studies have

reported that they are concentrated in limbic structures, areas of the forebrain including the septum, the paraventricular nucleus of the hypothalamus, the anterodorsal thalamic nucleus, and in discrete regions of the midbrain and hindbrain (v.g., dorsal raphe, substantia nigra, locus coeruleus) (Guitart, Codony, & Monroy, 2004). From a behavioural point of view these receptors have been implicated in depression (Bermack & Debonnel, 2005; Kulkarni & Dhir, 2009; Sabino et al., 2009), pain (Cendan et al., 2005) or aggression (Beltrán, Cavas, & Navarro, 2006), as well as in reinforcing effects of different drugs (Beltrán, Cavas, & Navarro, 2004b). Recently, the role of sigma receptors, specially the sigma-1 receptor subtype, has been identified as a target for the pathophysiology of neuropsychiatric disorders, including anxiety. However, the preclinical and clinical evidence is meager at present. Sánchez et al., (1997) found that Lu 28-179, a selective sigma-2 ligand, facilitated the exploratory behaviour of mice and rats in the black and white two-compartment box, suggesting an anxiolytic-like effect in rodents. Likewise, opipramol, a high-affinity nonselective sigma-receptor agonist, is active in several behavioural paradigms

indicative of anxiolytic properties at doses (1-10 mg/kg) (Müller, Siebert, Holoubek, & Gentsch, 2004). This drug has been effective in patients suffering from anxiety disorders, although is not clear whether the actions on anxiety are mediated by sigma receptors since opipramol also possesses histamine-H1 antagonist properties. On the other hand, sigma-receptor agonists, such as (+) SKF 10,047 attenuated the conditioned fear-stress-induced anxiety response in rats (Noda, Kamei, & Nabeshima, 1999). However, to our knowledge, there is no information with respect to the possible effects of sigma-1 ligands on anxiety behaviour in classical animal models. Consequently, the present study examined the effects of (+)SKF 10,047 (2, 4 and 8 mg/kg, i.p.), a sigma-1 prototypical agonist, on anxiety tested in two laboratory models in mice (elevated plus maze and social interaction test).

## Methods

### Subjects

A total of 120 albino male mice of the OF.1 strain (provided by CRIFFA, Barcelona, Spain) weighing 25-30 g were used. Animals were housed in groups of five in plastic cages (24 × 13.5 × 13 cm) under standardized lighting conditions (white lights on 20:00-8:00), a constant temperature (20 ºC), and food and tap water available *ad libitum*, except during behavioural tests. Cage maintenance was undertaken twice weekly, but never on the day of testing. Mice were housed 7 days before the experiment.

This experiment was carried out in accordance with the Guiding Principles for Care and Use of Laboratory Animals approved by the European Communities Council Directive of November 24, 1986 (86/609/EEC).

### Procedure

#### Drug administration

Four groups of animals were used in each experiment. Animals were randomly allocated to one control group (n= 15) receiving physiological saline and three experimental groups (n= 14-17 each) receiving (+)SKF 10,047 injections (Tocris Laboratories). Drug or vehicle was injected intraperitoneally in a volume of 10 ml/kg. Tests were performed 30 min after injections.

#### Behavioural test

Experiment 1 (Social interaction test)

The general design of the social interaction test was adapted from File (1980). A pair or mice was confronted in a familiar area, weakly lit by a red 40 W bulb. This experimental situation facilitates social interaction between animals, thus allowing one to easily examine the action of anxiogenic/anxiolytic compounds. The social encounters were videotaped using a Sony V8 camera. The tapes were analyzed using a microprocessor and a custom-developed programme (Brain, McAllister, & Walmsley, 1989), which facilitated estimation of time allocated to 10 broad behavioural categories. Each social interaction session lasted for 5 min. The names of the categories as follows: body care, digging, non-social exploration, exploration from a distance, social investigation, threat, attack, avoidance/flee, defence/submission and immobility

(Navarro & Manzaneque, 1999; Navarro & Pedraza, 1996). The analysis was done by a trained experimenter who was unaware of the treatment administered to the groups.

Experiment 2 (Elevated plus maze test)

Plus-maze test consisted of two open arms (30 × 5 cm, surrounded by a 0.25-cm-high border) and two closed arms (30 × 5 cm, surrounded by 15-cm-high walls) with the two pairs of identical platform, which emerged from a central platform (5 × 5 cm), positioned opposite each other. The apparatus was elevated 40 cm above the floor. Mice were tested on the maze in randomized order. The test was initiated by placing the mouse on the central platform of the maze, facing one of the open arms, and letting it move freely. Each session lasted 5 min, being recorded by a videocamera. All tests were carried out under dim red lighting between the second and seventh hour of the dark phase. After each test, the maze was thoroughly cleaned. Behavioural analysis was performed by a trained experimenter who was blind to treatment condition. A number of classical parameters were collected during the session: (a) Open arm duration: the total amount of time the mouse spent in the open arms; (b) Closed arm duration: the total amount of time the mouse spent in the closed arms; (c) Central platform duration: the total amount of time the mouse spent in the central platform; (d) Open arm frequency: the frequency of mouse entry with all four paws into the open, unprotected arms, and (e) Total number of entries in the arms.

### Data analysis

Nonparametric Kruskal-Wallis tests were used to assess the variance of the behavioural measures over different treatment groups. Subsequently, appropriate paired comparisons were performed using Mann-Whitney U tests to contrast the parameters in the different treatment groups. The analyses were carried out using nonparametric statistics since the criteria for parametric statistics were not met by the data. A value of $P < .05$ was considered to be statistically significant.

## Results

### Experiment 1

Table 1 illustrates medians (with interquartile ranges) of each behavioural category analyzed. Kruskal-Wallis analysis showed that there were significant differences in non-social investigation ($p < 0.005$) and social investigation behaviours ($p < 0.05$). Paired comparisons using Mann-Whitney U tests revealed that, as compared with the control group, mice treated with (+)SKF 10,047 (8 mg/kg) showed a significant reduction in total time spent in social investigation behaviours ($p < 0.005$) whereas non-social investigation behaviours were significantly increased ($p < 0.05$).

### Experiment 2

Table 2 illustrates medians (with interquartile ranges) of duration and number of entries in all the different areas of the maze. Kruskal-Wallis analysis showed that there were significant differences in the number of entries and the time spent in open arms ($p < 0.05$). Paired comparisons using Mann-Whitney U tests revealed that (+)

SKF 10,047 (4 and 8 mg/kg) produced a significant decrease in the time spent in open arms as well in the number of entries in this area, as compared with the control group ($p < 0.05$-$p < 0.005$).

**Table 1**
Median values (with interquartile ranges) for total time (sec) in each behavioural category analyzed of the "social interaction test" in animals treated with (+)SKF 10,047

| Behavioural categories | | Doses of (+)SKF 10,047 | | |
|---|---|---|---|---|
| | Control | 2 mg/kg | 4 mg/kg | 8 mg/kg |
| Body care | 6.8 (0-59) | 5 (0-27) | 4.9 (0-34) | 10.3 (0-40) |
| Digging | 0.4 (0-5) | 1.1 (0-14) | 6.1 (0-10) | 0 (0-19) |
| Non-social ## exploration | 230 (135-278) | 261 (167-285) | 241 (197-279) | 268 (240-285)* |
| Exploration from a distance | 5.3 (0.7-45) | 4.2 (0-13) | 6.8 (1.4-26.5) | 6.1 (1-19) |
| Social # investigation | 34.5 (3-79) | 19 (4.7-132) | 28.5 (0-79) | 10.7 (1.4-45)** |
| Threat | 0 (0-11) | 0 (0-1) | 0 (0-9.4) | 0 (0-0) |
| Attack | 0 (0-10) | 0 (0-0) | 0 (0-4.4) | 0 (0-0) |
| Avoidance/Flee | 0 (0-3.6) | 0 (0-9.4) | 0.4 (0-16) | 0 (0-7.6) |
| Defence submission | 0 (0-39) | 0 (0-2.5) | 0 (0-66) | 0 (0-5.4) |
| Immobility | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) |

Kruskal-Wallis test showed significant variance, # $p < .05$; ## $p < .005$
Differs from controls on Mann-Whitney U tests, * $p < .05$; ** $p < .005$

**Table 2**
Median values (with interquartile ranges) of behavioural measures in the elevated plus maze test in animals treated with (+)SKF 10,047

| Treatment | Doses (mg/kg) | Duration (s) | | | Frequency | |
|---|---|---|---|---|---|---|
| | | Open # arms | Closed arms | Center | Open arms# | Total entries in the arms |
| Saline | 0.0 | 48 (0-288) | 142 (0-200) | 100.5 (12-143) | 3.5 (0-7) | 12 (2-20) |
| Drug range | 2 | 35 (0-210) | 158 (45-235) | 79 (27-200) | 2 (0-12) | 13 (3-21) |
| | 4 | 16 * (0-128) | 168 (0-246) | 105.5 (54-300) | 1 * (0-10) | 10.5 (0-18) |
| | 8 | 0 ** (0-290) | 131 (0-231) | 152 (10-300) | 0 ** (0-6) | 9 (0-18) |

Kruskal-Wallis test showed significant variance, # $p < .05$
Differs from controls on Mann-Whitney U tests, * $p < .05$; $p < .005$

## Discussion

In the present study, we analyze for the first time the effects of (+)SKF 10,047, a sigma-1 prototypical agonist, on anxiety tested in two classical animal models of laboratory (social interaction test and elevated plus maze test). The social interaction test of anxiety has been proved extremely useful in detecting both anxiogenic and anxiolytic effects of drugs. An increase of social interaction without a concomitant increase in motor activity is indicative of an anxiolytic effect, whereas a specific decrease in social interaction indicates an anxiogenic effect (File & Seth, 2003; Navarro et al., 2004). Acute treatment with (+)SKF 10,047 (8 mg/kg) produced a significant decrease of social investigation, without affecting immobility. This behavioural profile could suggest the existence of a slight anxiogenic-like activity of (+)SKF 10,047 in mice. The rest of the behavioural categories analyzed were not significantly modified by the drug, except for a mild increase of non-social exploratory behaviours observed with the highest dose of (+)SKF 10,047.

Exploratory behaviours in novel environments is the basis for many psychopharmacological tests of anxiety, including the elevated plus maze. This test is routinely used to study anxiety-related behaviours in rodents (Lister, 1987). In this situation, mice will show a pattern of behaviour characterized by open-arm avoidance. This tendency is suppressed by anxiolytic and potentiated by anxiogenic agents (Navarro, Luna, & García, 2005; Navarro, Burón, & Martín-López, 2006). Results showed that (+) SKF 10,047 (4 and 8 mg/kg) produced a significant reduction in the number of entries in open arms, as well as in the time spent in this area, as compared with the control group, without depressing motor activity. Overall, these results indicated that (+)SKF 10,047, in concordance with the findings obtained in the experiment 1, also exhibits an anxiogenic-like profile in the elevated plus-maze test in male mice.

Few studies have investigated the role of sigma-1 receptors in anxiety, with conflicting results. (+)SKF 10,047 attenuated the conditioned fear stress-induced anxiety response, which is not attenuated by typical anxiolytics or antidepressants (Noda et al., 1999). Sigma-1 receptor 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 (Sabino et al., 2009). By contrast, Chevallier, Keller, & Maurice (2011) found recently that male sigma-1$^{-/-}$ mice showed signs of anxiety in the open-field, passive avoidance or elevated plus-maze test.

The behavioural effects on anxiety found in this study could be related to the known ability of sigma-1 receptor for modulating different brain neurotransmitter systems implicated in anxiety regulation such as 5-HT (Bermack & Debonnel, 2001), acetylcholine (Graef, Schönknecht, Sabri, & Hegerl, 2011) or dopamine (Karasawa, Takahashi, Takagi, & Horikomi, 2002) (for a review, see Beltrán & Navarro, 2007). For instance, there is a strong relationship between sigma-1 receptors and dopaminergic neurotransmission, and sigma-1 receptors are present in dopamine neurons. In fact, (+)SKF 10,047 increases the firing rate of dopamine neurons, suggesting that sigma-1 receptors are modulating the dopaminergic system (Karasawa et al., 2002). The cholinergic system has been also connected to anxiety. In this sense it has been demonstrated that sigma-1 receptor agonists themselves interact with the cholinergic system in different brain areas involved in anxiety regulation (Graef et al., 2011). On the other hand, the effects of sigma-1 ligands on anxiety also might be related to their ability to modulate other neurotransmitter systems such as nitric oxide or delta opioid receptors. For example, it has been suggested a functional interaction between sigma receptors and nitric oxide (Mamiya et al., 2000). Likewise, rubiscolin-6, a

delta opioid peptide, is known to possess actions on anxiety via sigma-1 receptors (Hirata et al., 2007).

The majority of clinical trials with sigma-1 receptors ligands have been carried out to explore their effect in the treatment of schizophrenia. However, some of the clinical trials exploring the protective effect of sigma-1 receptor ligands in major depression and anxiety are also ongoing. For example, igmesine and opipramol, both sigma-receptor ligands, have already been shown to be beneficial in patients suffering from these psychiatric disorders, although their therapeutic actions probably may be mediated by other neurotransmitter systems (Kulkarni & Dhir, 2009).

In conclusion, the results of the present study indicate that sigma-1 agonist (+)SKF 10,047 produced anxiogenic-like effects in two laboratory animal models in mice. This action on anxiety probably is related to its ability to modulate other neurotransmitter systems. Further studies are required to better establish the role of sigma-1 receptors in anxiety regulation.

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