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EXPERIMENTAL STUDIES ON FRUSTRATION: BEHAVIORAL, PHYSIOLOGICAL AND PHARMACOLOGICAL APPROACHES IN RATS

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A mi sobrinita Alicia,

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Introduction

1. Effect of previous experiences on the emotional response

"Man is by nature a social animal; an individual who is unsocial naturally and not accidentally is either beneath our notice or more than human. Society is something that precedes the individual. Anyone who either can not lead the common life or is so self-sufficient as not to need to, and therefore does not partake of society, is either a beast or a god"

Aristoteles

As Aristoteles said, human beings tend to live in a politically organized society. Notwithstanding the above, social relationships are complicated due to conflict of interest between the parts. Our social relationships have a direct impact on our quality of life, as well as on our self-esteem, feeling of control and ability to adapt (Hardin & Higgins, 1996). All of these depend on the people which create and maintain the experience of their reality in a process of social verification. There is no black or white in society, our reality is subjective so that the response or feeling depends on the individual.

Conflicts are part of daily life in the contemporary world which includes reduction of salaries, dismissals, divorces, social exclusion, among other aspects (Kamenetzky et al., 2009). For the sake of argument, a person wants to propose marriage to his partner but unfortunately, he received a negative response. Another example occurs when a person that is close to us dies or when someone is fired from their job. These examples encompass several circumstances which provoke a violation of expectations. These types of situations involve a loss, either total or partial, of an expected positive reinforcer that causes a kind of pain from sensory stimuli but which, however, can be interpreted as a kind of pain not caused by a physical blow (Mustaca, 2018; Papini et al., 2015).

These authors suggested that there are a set of circumstances which can induce emotional responses and, likewise, these responses could be swayed by previous expectancies, personality or context. Accordingly, the frustration is a highly studied emotional response and it will be the focal point of this Thesis.

Social exclusion, divorce or a failure of some sort are situations in which the reward obtained is lower than expected, being also a sudden loss. This loss or unexpected devaluation of the reward triggers a series of aversive behavioral, physiological and cognitive changes that are known as frustration (Papini, 2009).

Frustration is an unpleasant feeling that occurs when someone gives all their physical, phycological or social efforts towards a set target, and despite this, it is not obtained. Studies on frustration started in 1928 with Capuchin Monkeys. Firstly, Tinklepaugh (1928) called (identified) it as a type of "disappointment" or "apparent anger". He indicated that when the reinforcement was replaced with another, which was of a lower quality or quantity, a set of behaviors of seeking and hesitation appeared . That suggested that there is a mental representation of the reward which sways the behavior. Subsequently, Dollard et al. (1939) considered it as a stimulus or interference between a sequence of responses that are directed towards a goal.

However, some years before this suggestion, the Behaviorism Age was in its last years. Behavioral theorists such as Thorndike (1911) and Hull (1943), based on the classical theories of reinforcement (stimulus-response associations are formed when an individual learns that the reinforcement strengthens such associations and punishment diminish that association), suggested that a greater reward produces stronger associations that it have been reduced when the punishment or frustration appear.

In the 1950s, there was an increase in emotional research and how emotion can influence behavior. Specifically, in the area of frustration. Brown and Farber (1951) , suggested two main ways in which frustration can affect behavior, as a motivational or as a learning process. The first one suggests that frustration can increase the level of motivation whereas the other way suggests that frustration is an internal stimulus which provokes a set of new responses. These consequences are acute initially due to the fact that they are present at the moment of frustration. However, other authors such as Yates (1975) tried to study how the effect of extinction provoked by frustration could affect in personality. He suggested that an "overlearned", understood as a high frequency of reinforcement associated with behavior, trigger the higher intensity in the state of frustration. Considering Yates (1975) contributions, the tolerance to frustration could indicate a higher acceptation of the downshift and, thus, different profiles of responses in conflictual events.

Clinical research has indicated that the continuous frustration experience could lead to the development of anxiety disorders such as obsessive-compulsive disorders, panic attacks as well as closely related depression, substance abuse or gambling among others (Harrington, 2006). Subsequently, Gray (1981) suggested a neural perspective similar to Yates contemplations. He considered that frustration and fear have the same neurobiological basis. In the Reinforcement Sensitivity Theory (RST) exposed, he determined a behavioral inhibition and activation system, BIS and BAS respectively. BIS is reactivated by punishment or non-reward response and BAS is reactivated by reward or non-punishment response. BIS has been related to depression, substance abuse disorder or antisocial personality disorder which suggests that a non-reward situation or a punishment could lead to a psychiatric disease. Similarly, he considered that the exposition to BIS and BAS alternatively produce a higher frustration tolerance which is called "resilience".

On one hand, Gray suggests that deficit of the reward could lead to an aggressive behavior common in disorders such as antisocial personality disorder (Blair, 2010). On the other hand, the high sensitivity to the punishment could lead to low state of mind common in anxiety disorders or depression (Gray, 1981). Gray's findings were in the pharmacological field which will be explain in point 2.2.

In 1992, Amsel defined frustration as we know it today. Thus, it was defined as an emotional state that occurs with the decrease or omission of the quantity or quality of an expected appetitive reward. In the same way, Amsel (1992) pointed out frustration as an experience which is not present in all people because of the individual interpretation of this reward devaluation. Amsel interpreted this reaction as an aversive unconditioned response called *primary frustration* of motivational nature. This definition includes when someone knows that their expectation has been violated, for example, the exact moment when you realize that the reward has been violated (Amsel, 1958; Amsel & Roussel, 1952). After that, it starts a conditioned response called *secondary reaction* of anticipatory nature in order to adapt to the new reward. Finally, there is a natural adaptation to the new context. When there is not an adaptation, some disorders appear as mentioned before.

According to Amsel (1958), there is a reaction to the frustration in the subject which depends on the context or conditions which have produced it. Amsel suggested that the reaction to the frustration has an initial increase in motivation (which can be

measured in speed of response) and a reduction or removal of that response. Amsel (1992) considered that motivation is also an important part of frustration. Motivation could be defined as a mental process which arouses or stimulates an organism to a determinate action (Amsel, 1992). It may be influenced by physiological drives or by external stimuli. For example, getting a promotion is what motivates someone to work hard. For this reason, motivation is usually a variable in frustration in such a way that higher expectations lead to higher frustration (Amsel, 1992). This suggested a response that Amsel (1958) considered, it was subsequently studied by Yates. Yates (1978) considered that frustration provokes a set of responses which starts with aggression, followed by fixation, regression and, finally, conflict. However, and according to Yates (1978) contributions, there are differences between the individuals. That is, two people don't respond to certain situations in the same way. The differences may be due to personality (the frustration tolerance profile) or to differences in context.

Firstly, Elliot (1928) had studied the importance of the context in the "expectation" that the animal had been created. Elliot (1929) suggested that a change in the environment provoke a set of responses in order to regulate or coordinate the behavior with the new environment. In an experiment with rats (*rattus novergicus*), he created two groups, an experimental and a controlled group. First, the experimental group was thirsty and rewarded with water for nine days (the animal has access to food *ad libitum*); later, in the following days, the animals were hungry and rewarded with food (the animal has access to water *ad libitum*). At this moment, the animal had water *ad libitum*. The controlled group was always thirsty and rewarded with water. The experimental group had a change in its context and, this change of conditions (hungry instead of thirsty) caused an increase in the average of errors and time of the experimental groups which, according to Elliot (1929), could be due to the "expectation" of the animal.

In the context of reward devaluation, two interpretations have been made. Capaldi & Molina (1979) suggest that the change in the behavior of the animal may be due to the memory of the reward before the devaluation. Specifically, Capaldi (1966) suggested a "sequential theory" and argued that frustration was a cognitive process which was regulated by memory. The author proposed that the memory lead to an operant conditioning which was interesting for frustration caused by partial reinforcement. On the other hand, Hulse (1980) suggests that the animal learns that the

behavior and the reward have a relationship which may allow the animal to extrapolate that certain behavior will cause a reward

In a possible conjugation of the two interpretations commented above, Papini (2003, 2006) suggested that when the sudden omission of the reinforcement occurs, there is a behavioral adjustment which depends on two types of learning processes. An *allocentric process* related to the knowledge of the environment and the adjustment of the behavior with respect to this environment by the subject. And on the other hand, an *egocentric process* that allows the organism to learn from its own emotional reaction, while acquiring the ability to anticipate it (Papini, 2003). In line with the other authors, Papini (2003) suggests that the sudden omission triggers an evaluation of changes in the conditions, which have produced the frustration, and perform an adaptive behavior. Simultaneously, there is an emotional response which alarms the body that something is not going well. This theory considers the main two points in frustration, the emotional and the learning process involved in frustration.

In this point, the definition of frustration has been changing to frustration or paradoxical effects of reinforcement (Amsel, 1992) to negative contrast or relativity of reinforcements (Flaherty, 1996) or surprise omission of reinforcers (Papini, 1997, 2003). However, all authors' theories point out the existence of two transitory processes with different mechanisms, one presented during the frustration and another one presented some time later (Amsel, 1992; Papini, 2003). These processes have been associated with behavioral, emotional, psychophysiological and neural stress responses (Papini et al., 2015).

1.1. Animal models of frustration

The study of frustration has been developed mainly with animal models such as rodents (Justel et al., 2012, Ortega et al., 2013) because of the behavioral and physiological similarity with the patterns in humans. Despite Aristoteles philosophy, animal behavior is such, and quite similar to that in humans. For example, Scarf et al., (2011) found that pigeons have a similar gamble behavior whereby they prefer higher risk for a higher reinforcer instead of a secure reinforcer.

The typical instruments used in the measurement of frustration are mazes, conditioning boxes, open field or social privation, among others (Flaherty & Rowan,

1986). Similarly, the typical reinforcer used is sucrose (solid or liquid) in different quantities or qualities between other qualitative reinforcers as social environment.

For the study of frustration, animal models could involve the total omission of an appetitive reinforcement in the extinction (Adelman & Maatsch, 1956), the reduction in reinforcement of its quality or quantity such as in successive negative contrast (Pellegrini et al., 2004; Pellegrini & Papini, 2007), the partial reinforcement in the partial-reinforcement extinction effect (Amsel, 1967), the change of a preferred reinforcement to another which was non-preferred in anticipatory contrast (Flaherty, 1996) or the impossibility of obtaining the reinforcer because of a physical barrier which was not there before (Mustaca, 2018). However, the most common procedure for studying frustration has been the Successive Negative Contrast (Papini, 2009).

1.2. Successive negative contrast

The Successive Negative Contrast (SNC) is a learning phenomenon related to two phases: a preshift phase and a postshift phase. In this phenomenon, an experimental group has gone through a situation of devaluation in the quantity and or quality of the reinforcer in the first phase of preshift to the second phase of postshift. The experimental group is compared to a controlled group which has always had a lower reinforcer (Figure 1).

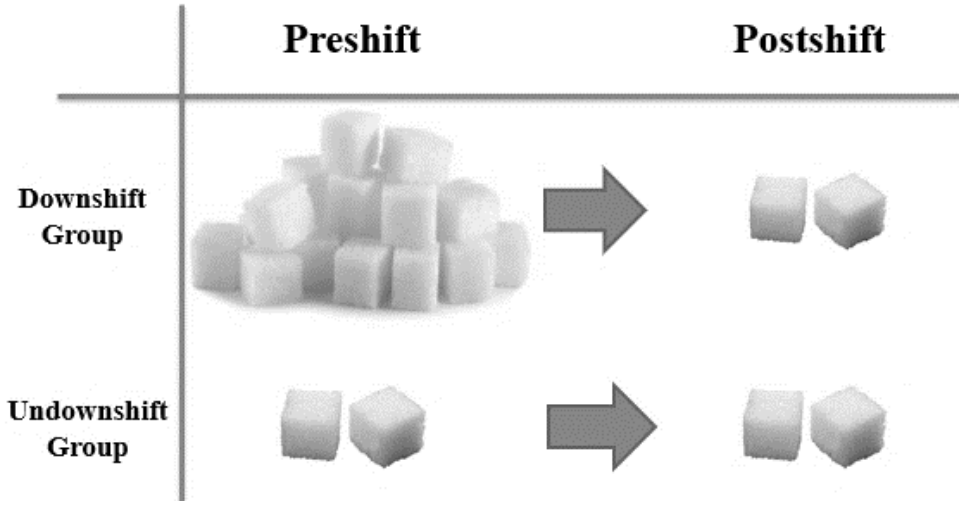

Figure 1. Theorical representation of SNC has been broken down into two phases: preshift and postshift phase, understood as before and after frustration. Similarly, it has been separated into two typical groups: Downshift and Undownshift groups. The Downshift group has a high reinforcement during preshift phase, but it has been reduced during postshift phase. It is compared with the controlled group or undownshift group which always has the lower reward.

The SNC procedure presents two major modalities depending on the accessibility of the reinforcers: instrumental or consummatory (Flaherty, 1982). Thus, when these reinforcers are directly accessible for the subjects, it is called consummatory SNC (cSNC). however, when it requires an instrumental response to obtain the incentive, it is called instrumental SNC (iSNC) (Figure 2).

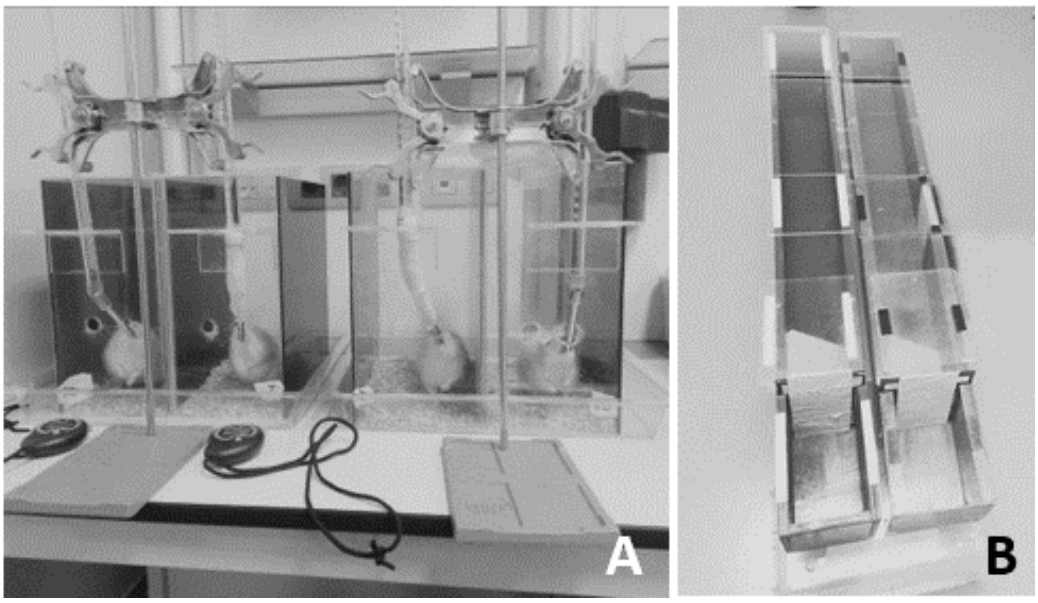

Figure 2. Instruments used typically in cSNC (A) and in iSNC (B). Figure 2.A shows four consummatory chambers with the rats doing the consummatory task. Sucrose is administrated in liquid form inside the burettes. Figure 2.B shows two mazes where the animal does the instrumental task. The animals are situated at the beginning of the maze and they have to run through the maze to achieve the reinforcer, which is situated in solid form (pellets) at the end of the maze.

This protocol has the main feature of inducing the frustration as previously explained in animal models. The preshift phase includes the environment and the circumstances that will induce the expectancy in the animal, that is to say, the animal will have the expectancy of a high reward (downshift group) or a low reward (undownshift group). Therefore, postshift occurs when the expectation has been violated. In this case, the devaluated animal was waiting for a high reward and, when it does not appear, a set of physiological, neural, hormonal and behavioral responses are triggered, and this is called frustration (Amsel, 1992). On the other hand, the unshifted group has not yet received any other reward and, because of this, they do not suffer a change in their behavior (Figure 3). This finding contradicts the classical theories of reinforcement of Thorndike (1911) and Hull (1943) due to the devaluation of the reward reduces the behavior to lower levels than the controlled group, even although both groups have the same reward.

Both instrumental and consummatory show a negative contrast under a reinforcement reduction. However, there are some differences between these two procedures. The measurement is one of the most important variances due to the fact that the iSNC is measured by the time it takes to cross the maze or runway, while the cSNC is measured by the sucrose intake. Thus, iSNC is provoked by a reduction in quantity whereas cSNC is provoked by a reduction in quality of the reinforcer.

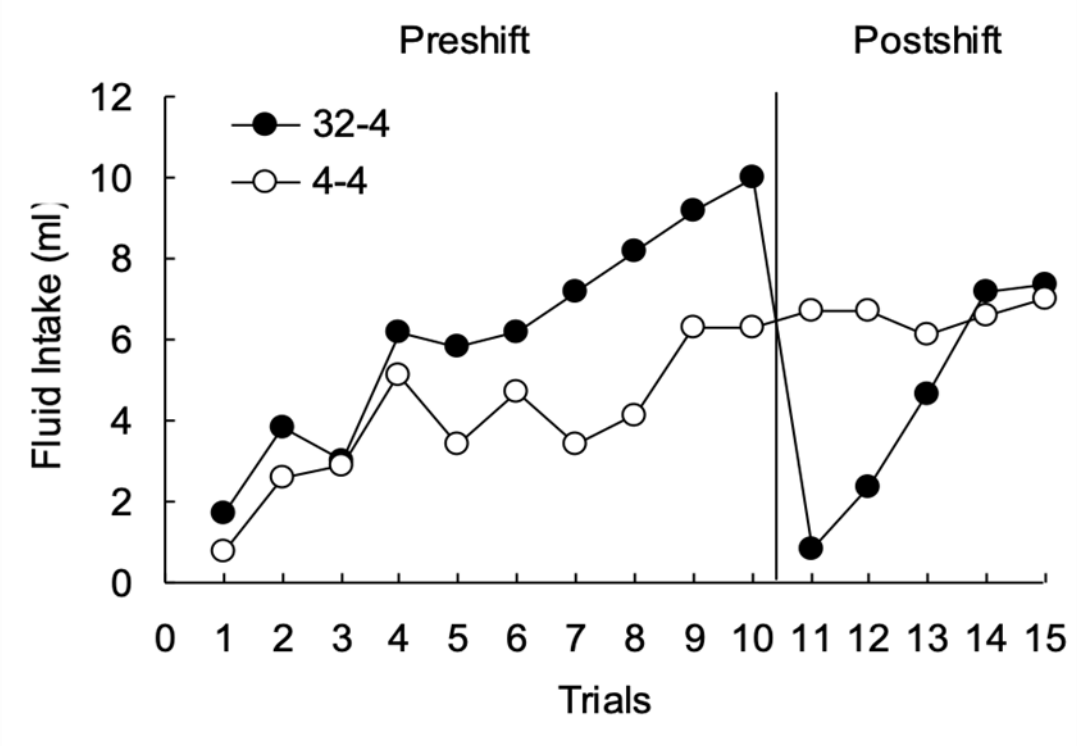

Figure 3. Representation of typical results obtained in a cSNC. The fluid intake, has been represented (ml) of 4% or 32% sucrose during the trials, separated by a line in preshift and postshift phase. The downshift group, represented as black circles, shows an increase of fluid intake during preshift which significantly decreases during postshift phase following the reward reduction. The Undownshift group, represented as white circles, shows a low incrementation during the trials and which has not any change between phases (Source: Papini, 2009)

1.2.1. Comparative perspective in Successive Negative Contrast

Firstly, the *species differences* in the SNC paradigm has been extensively researched in animal models such as, pigs (Luo et al., 2018), dogs (Dzik et al., 2019) and mice (Mustaca et al., 2000), but the common model has been practiced in rats. An important point to keep in mind about SNC was the presence of frustration in fish (Breuning et al., 1977), turtles (Pert et al., 1974) and marsupials (*Lutreolina crassicaudata* y *Didelphis albiventris*) (Papini et al., 1988). However, SNC did not occur in the instrumental procedure in goldfish or turtles (Papini & Ishida, 1994; Pert & Bitterman, 1970). Under

these conditions, there was a tendency but not a significant change in the behavior which could suggest a contrast. Differences were found in the consummatory contrast in several species.

Frustration, measured by SNC, suggests the existence of a basis mechanism that evolved from a common reptilian ancestor (Bitterman, 1988) which could be an emotional reaction or a sensory adaption (Papini et al., 1988).

The most common model used in experimental research is the rat model which *no strain differences* have been found (Flaherty et al., 1978; Torres et al., 2005). It suggests that, regardless of that fact, the frustration effect appears. However, the frustration can be altered considering some selected breeding strain. The SNC is a group phenomenon with individual differences that are not a cause of the strain. The selective breeding consists in separating higher drinkers (or lower drinker) and pairing them with each other during seven generations. The breeding selection showed differences in the activity which means a large or small contrast, but these results did not correlate with the contrast (Flaherty el al, 1994). Alternatively, it was found that behavioral divergences between the Roman high- (RHA) and low- (RLA) avoidance rats in iSNC (Rosas et al., 2007) but not in cSNC (Gómez et al., 2009). Low avoidance rats looked more fearful than high, suggesting that SNC requires an emotional mechanism which is sensitive to some selective breeding.

The topic of *sex* and *developmental differences* has also been studied by Flaherty et al. (1994). Initially, there is not any difference between female and male, but female rats showed a larger contrast when the shift was from a sucrose solution to a quinine-adulterated solution (Flaherty & Rowan, 1989). To the contrary, the developmental differences have suggested that younger rats (18-days-old) discriminate the reward in a consummatory contrast (Stanton et al., 1984; Suarez et al., 2014), but do not show frustration in an instrumental contrast in 25-day-old rats (Roberts, 1966).

1.2.2. Parameters in Successive Negative Contrast

When there is a violation of the expectations, there is an abrupt change in the behavior that tends to regulate itself during these days. This abrupt change in the behavior has a great influence of context (Capaldi & Molina, 1979; Flaherty et al., 1983) and personal differences (Yates, 1978) which could induce alterations in response against a reward devaluation.

Studies have shown the influence of emotional mechanisms or context in the reward devaluation. Consequently, although the contrast is well defined today, several investigations tried to determinate the parameters to find the higher contrast (Flaherty, 1996).

Reward disparity

The disparity has been a point of interest because the reinforcer effect of the sucrose determines the appearance of the contrast. Authors suggest that the initial natural response to the reward devaluation is "*Where is my old reward?*", that is, the animal is looking for the reinforcement obtained in preshift phase. Thus, the disparity has to be large enough to notice that difference. iSNC has found a greater contrast when animals compare a ratio of 1:16 units (Crespi, 1942) while in cSNC appears when it compares a ratio of 1:8 units (Pellegrini & Papini, 2007). Similarly, and due to cSNC it requires a quality devalue of the reinforcer, a shift from sucrose to saccharine solutions showing a large and enduring contrast (Flaherty, 1996; Pellegrini et al., 2004).

Deprivation

SNC usually implies a reduction of the daily chow to their free-feeding weight (82-85% *ad libitum*). Capaldi (1971) focused on the deprivation for its potential to affect the reward expectancy.

iSNC have shown a reduction or elimination of the contrast if the deprivation is higher than 90% *ad libitum* (Flaherty & Kelly, 1973) or when the deprivation is enhanced in the shift day (Capaldi, 1971). However, this effect disappears when there is a shorter inter-trial interval (ITI) or more trials per day (Capaldi and Sight cited by Flaherty, 1996).

On the other hand, cSNC appears whatever the level of deprivation. Furthermore, the time of recovery is greater in non-deprived rats compared to deprived rats. The administration of insulin during the shift day suggest a loss of calories during the deprivation which need to be recovered with the sucrose intake (Drobes et al., 2001; Flaherty et al., 1983).

Time of access

The time of access involves the session time and the number of sessions. Pellegrini et al. (2004) pointed out that frustration seems to be greater in those animals trained in a greater number of sessions of shorter duration. Specifically, animals recovered faster in the sessions of 10 minutes compared to the typical sessions of 5 minutes.

The retention interval between the last experience of preshift reinforcer and the first experience of postshift has been also determined. SNC appears even after 24 days after preshift phase in iSNC (González et al., 1973) and marginally after 17 days in cSNC (Flaherty and Lombardi, 1977). However, the contrast is reduced as time passes and completely eliminated after 68 days in iSNC (Gonzalez et al., 1973) and after 30 days in cSNC (Flaherty & Lombardi, 1977).

Prior reward experience

The continuous experience to the lower reinforcer in the experimental group reduces the contrast in both procedures (Flaherty, 1996). The partial reinforcement seems to reduce or retard by prior experience to partial reinforcement in cSNC, that is to say, a variated experience to the higher experience and a non-rewarded experience (water) during the preshift phase (Flaherty, 1996; Pellegrini et al., 2004). Similar results were found in RHA and RLA in an iSNC procedure when RHA did not show contrast and RLA showed an attenuated contrast (Cuenya et al., 2012).

Flaherty et al. (1983) tried to study how a change in the animals' expectations may induce different responses. In this experiment, Flaherty et al. (1983) created three groups: an experimental group with a high reward, which will be devaluated later, a rising reward group, which reward will be devaluated as well, and finally, a controlled group with non-devaluation of the reward. He considered the two interpretations mentioned previously and, consequently, suggest two possibilities to the animal behavior. First, the animal may do an average of rewards before the devaluation and it would create an expectation considering that average (McHose & Peters, 1975). Secondly, the animal may create an expectation considering previous experiences of reward incrementation and, likewise, its anticipation would be frustrated during the reward devaluation. In this interpretation the expectation for the next session was higher that when the animals received the same reward daily.

In this experiment, Flaherty et al. (1983) didn't find differences between the rising reward group and the experimental group and, for this reason, the first interpretation of and averaging response was accepted (Figure 4). However, the authors suggested that the type of reward (solid or liquid in this case) could be essential in other results.

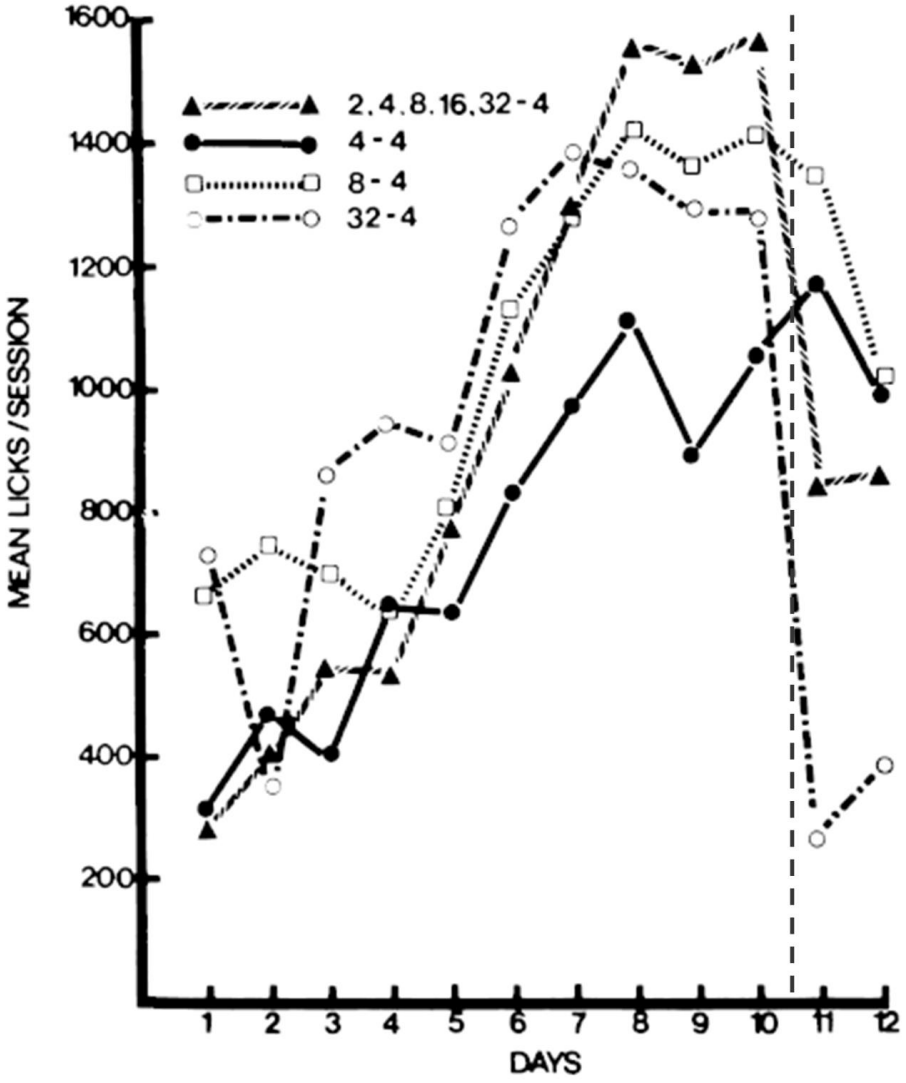

Figure 4. Figure represents the mean licks per session (axis Y) during the days (axis X) and considering the type of the devaluation. The rising reward group has been represented with the triangle group, the control group has been represented with black circle group and the experimental group with white circle. An experimental group with a lower reinforcement has been represented with the white square. Numbers in group indicate the percentage of sucrose per every 100 ml. Discontinuous line represents the moment of reward devaluation (Source: Flaherty et al., 1983).

Environmental variables

A context with a high number of distractions seems to reduce the contrast insofar the contextual stimuli hindered the learning and memory of rodents associated with the SNC (Flaherty, 1996). In consequence, in a non-enrichment environment the animals show a more perseverant frustration than animals exposed to an enrichment environment (Burman et al., 2008; Daniel et al., 2008; Mitchell et al. al., 2012). Other conditions, such as stress induced by restriction, increase the anxiety associated to the SNC for more days (Cuenya et al., 2012).

Authors have suggested that frustration involves two different process: a shocking non-emotional answer that is presented in the first postshift day and an emotional process when the animal associates the consummatory chamber or the maze with the aversion presented the day before, in the second postshift day (Flaherty, 1996). When the animal is under a non-enrichment environment, a readjustment of the behavior to the new reward will be more difficult, because of the negative emotional suffered under those conditions (Burman et al., 2008; Daniel et al., 2008; Mitchell et al. al., 2012).

2. Physiological basis of frustration

2.1.Frustration, pain and anxiety

David Livingstone (1857) was a Scottish missionary and doctor who explored Africa and recorded his travels in a book. In one of his explorations he was brutally attacked by a lion and, although he crushed his shoulder, he commented that he had not felt any pain during the attack. The way in which a stressful situation can alter the physiological response of the body has been studied extensively (Davis et al., 2004; Torres & Papini, 2016). For example, painful conditions, such as fibromyalgia, can lead to anxiety disorders arising from stress (Gracely et al., 2012) and, as Livingstone experimented, stressful situations could lead to a change in the pain tolerance. Some authors considered that it was a result of a particular part of the brain, the rostral cingulate cortex. That is an important area in the brain which has a key role in the processing of negative affect and motivation (Papez, 1937) as well as pain modulation (Vogt & Sikes, 2009) and cognitive control (Cole et al., 2009). In this context, Shackman et al., (2011) suggests a common function, between these processes, to

determinate the better performance in an uncertainly environment called adaptive control hypothesis (Shackman et al., 2011).

One of the responses involved in frustration is the release of cortisol or corticosterone in the organism caused by the activation of the hypothalamic-pituitary-adrenal axis (axis HPA). The HPA axis regulates several processes in response to stressful provocations which includes both physical and psychological stimuli that break the homeostasis or the internal balance (Engelmann et al., 2004, Martinez et al., 2007).

Consequently, the response associated with stressful situations will try to overcome the threat associated with new stressful situations in order to recover homeostasis. These responses are adaptive and characterized by activating the sympathetic nervous system, secreting adrenaline through neurons and chromatic cells of the adrenal medulla. Also, the release of adrenaline causes an increase in excitement, attention, alertness and vigilance (Engelmann et al., 2005).

Anatomically, the HPA axis is formed by the structural components: *the paraventricular nucleus of the hypothalamus* (PVN), which contains neuroendocrine neurons that synthesize and secrete vasopressin and CRH (corticotrophin-releasing hormone). In addition, these two peptides stimulate the secretion of corticotrophin (ACTH) in adenohypophysial corticotropic cells which in turn is performed in a specific area of the adrenal glands involved in the synthesis of glucocorticoids (GC). These glands consist of two parts: cortex and medulla. In the cortex they are divided into three layers, beginning from the outside to the inside the glomerular, fasciculate and reticular. The fasciculate cortex is involved in the GC synthesis, specifically cortisol and corticosterone. The adrenal medulla is composed of cells that act as neurons and the secretion of hormones is controlled by the sympathetic nervous system. The main hormones secreted by the adrenal medulla are adrenaline and noradrenaline (Douglas, 2005; Engelmann et al., 2005).

As previously mentioned, frustrating situations could lead to several psychiatric disorder such as depression, anxiety, substance abuse among others. Likewise, both depression and aggression are characterized by a low frustration tolerance (Blair, 2010; Deater-Deckard et al., 2010; Mahon et al., 2007). Dollard et al. (1939) points out frustration as a former criterion for agonistic responses such as aggression (Mustaca, 2018). Thus, an aggressive response, as well depressive, in a downshift reward situation

have been expected. It has been suggested that the exposition to continuous stressful situations in daily life could provoke an increase activity in HPA axis and corticotropin-releasing factor (CRF) circuits (Pariante et al., 2008; Sánchez et al., 2001). Studies have pointed out that hyperactivity in HPA axis could be not a consequence of psychiatric disorders, but a manifestation of a persistent neurobiological abnormality which lead to these disorders (Pariante et al., 2008). Additionally, authors have suggested that depressive or aggressive responses to frustration are not just dependent on personality, but conditions that could induce frustration (Sanchez et al., 2001). Moreover, intentional non-reward could lead to higher aggressive responses than those which are not on purpose (Dill & Anderson, 1995).

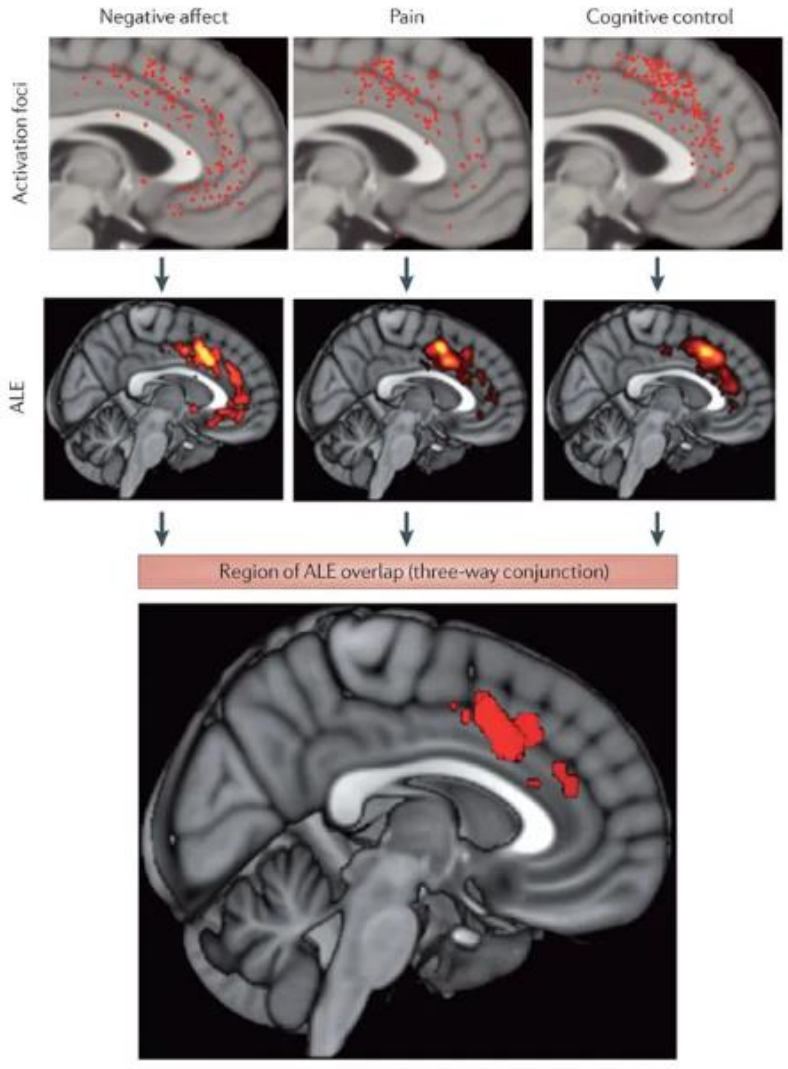

Figure 5. The image represents the activation map of negative affect, pain and cognitive control measured by a PET procedure. The map has been done according to 192 experiments (more than 3000 participants) obtained in Brain Map database. Authors suggest an overlap among three processes in the rostral cingulate cortex, according to the activation likelihood estimated (ALE) algorithm (Source: Shackman et al., 2011).

According to Shackman et al. (2011), there is an anatomical and physiological convergence between pain, negative affect and motivation (Figure 5). The anatomical integration suggests that the rostral cingulate cortex receives subcortical connectivity of dopaminergic pathways, from the substantia nigra and mediodorsal thalamus, the ventral tegmental area and the spinothalamic tract, and projects to the striatum (nucleus accumbens) and amygdala (Morecraft et al. cited in Shackman et al., 2011). It is the adaptive control hypothesis which suggests that the common function, between these processes, is the necessity to determine the better performance in an uncertainly environment (Shackman et al., 2011).

Due to the difficulties for designing an experimental research, frustration has not usually been studied in human models. However, some authors have tried to replicate the SNC in a "block of reward" task, such as Yu et al. (2014). Researchers found similar activation to frustration than Shackman et al. (2011). Yu et al. (2014) suggested that the dorsal anterior cingulate cortex or dACC (situated in the same place that the rostral cingulate cortex) network play an important role in frustration as well as aggression measured by fMRI (Figure 6). This activation of the cingulate cortex has also been found in animal models, which will be explained down below (Ortega et al., 2012).

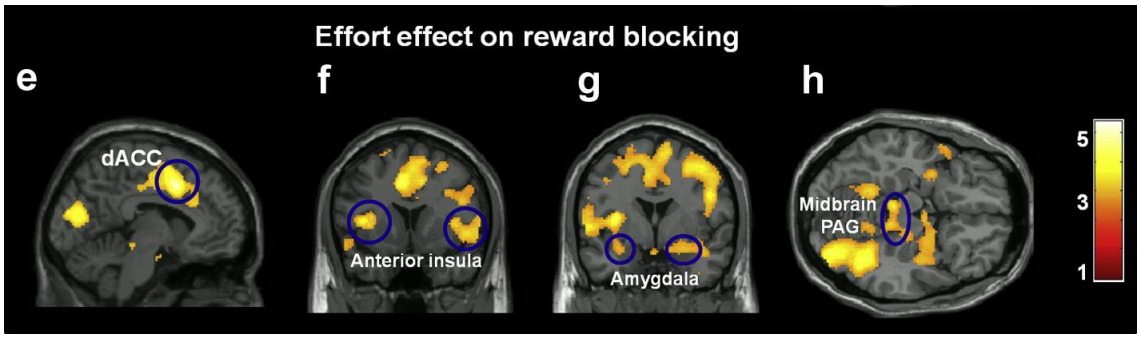

Figure 6. PET neuroimaging in a block of reward task. The results showed an activation in key). areas of animal models of aggression, including the left amygdala, left midbrain Periaqueductal Grey (PAG), left anterior insula, and dorsal anterior cingulate cortex (dACC) increased when the reward was blocked (Source: Yu et al., 2014)

The procedural and designing difficulties have focused the investigation of pain and frustration on animal models, specifically on SNC procedure. Mustaca & Papini (2005) found that frustration induces hypoalgesia (that is, a lower sensitivity to pain) after the second postshift session when it was measured by the hot plate. On the other hand, in other experiment, formalin was applied to the hind paw before the first postshift session (in order to induce physical pain through inflammation) enhances the cSNC, which

mean an incrementation of frustration throughout the postshift sessions (Ortega et al., 2011). All these findings support the adaptive hypothesis between physical pain and emotion.

Several data from neurogenetic studies have supported that expression of hippocampal genes are involved in the devaluation of rewards, being related to behavior disorders such as substance abuse or chronic depression, among others (Sabariego et al., 2013). In addition, the high expression of pCREB (phosphorylation of cAMP response element-binding), a marker of synaptic plasticity, in the prelimbic cortex, the anterior cingulate and the dorsal-medial striatum support the importance of memory consolidation in the reduction of rewards (Glueck et al., 2015).

The specific lesion of a part of the brain has supported the adaptive control hypothesis in SNC. However, there are differences between the instrumental and the consummatory procedure of SNC which are presented in the Table 1. The results in the literature revision suggest different process for iSNC and cSNC. Some of these differences could be due to differences of the procedure, as it was commented before.

It has been supposed and entorhinal – hippocampal- cingulate- striatal system to the instrumental contrast and the solitary tract- PBN-Hypothalamus and limbic system – amygdala to the consummatory contrast. Therefore, it is suggested that the iSNC could be more involved in the adaptive control hypothesis suggested earlier (Shackman et al., 2011).

Table 1. Differences in brain lesion of areas during consummatory Successive Negative Contrast and instrumental Successive Negative Contrast. Listing of brain lesion that have been investigated in successive negative contrast. The type of lesion has been added as well as the study which found that results.

	cSNC			iSNC		
	Author	**Type of lesion**	**Results**	**Author**	**Type of lesion**	**Results**
Amygdala	Becker et al. (1984)	Electrolytic	Lesions of the basolateral amygdala reduce contrast but lesions of corticomedial amygdala eliminate it	Salinas and White (1998)	-	A system that includes the amygdala may acquire a conditioned aversive response to the goal box after the shift is detected, leading to reduced speeds over testing.
	Liao & Chuang (2003)	Infusions of diazepam	Intra-amygdala infusions of diazepam significantly attenuated the consummatory negative contrast on the second postshift day.			
	Kawasaki et al. (2015)	Infusion of lidocaine	Centromedial amygdala inactivation reduced the cSNC effect			
Cingulate cortex	Ortega et al. (2011)	Electrolytic	Lesions in anterior cingulate cortex exhibited a significant retardation of recovery from cSNC relative to downshifted shams.			
Entorhinal cortex	Flaherty et al. (1994)	Aspiration lesions	No results found in cSNC	Flaherty et al. (1994)	Aspiration lesions	Aspiration lesions of the entorhinal cortex eliminated contrast but only in the goal section.
Gustatory thalamus	Reilly & Trifunovic (1999)	Electrolytic	SNC was eliminated after bilateral lesions of the gustatory thalamus.	Sastre & Reilly (2006)	Excitotoxic lesions	Gustatory Thalamus is not involved in iSNC following an unexpected reduction in value of a solid food reward.
Hippocampus	Liao & Chuang (2003)	Infusions of diazepam	Intrahippocampus infusions of diazepam do not attenuated the consummatory negative contrast.	Salinas and White (1998)	-	A neural system that includes fimbria–fornix is required to retain information about reduced reward over the 3-min ITI.

	Flaherty et al. (1998)	Excitotoxin ibotenic acid	Rats with damage to the hippocampus produced by the excitotoxin ibotenic acid does not influence in cSNC.	Flaherty et al. (1998)	Excitotoxin ibotenic acid	Rats with damage to the hippocampus produced by the excitotoxin ibotenic acid failed to show an iSNC.
Striatum				Salinas and White (1998)	-	One system that includes the dorsal striatum promotes a reinforced approach response to the goal box.
Nucleus Accumbens	Leszczuk & Flaherty (2000)	Electrolytic lesions	Lesions of nucleus accumbens don't influence in consummatory negative contrast	Leszczuk & Flaherty (2000)	Electrolytic lesions	Lesions of nucleus accumbens reduce instrumental negative contrast
Parabraquial nucleus (PBN)	Grigson et al. (1994)	Electrolytic lesions	Lesions of PBN prevent the cSNC compare the sham group.			
Prefrontal Cortex	Ortega et al. (2013)	Electrolytic lesions	Lesions of the ventrolateral orbital cortex reduced suppression of consummatory behavior after the incentive downshift, but only during the first downshift trial.			

2.2.Drug effects in Successive negative contrast

As it has been mentioned before, Livingston (1895) was attacked by a lion and he did not feel any type of pain. It is known that when one suffers a physical pain, it releases endogenous opioids in order to reduce that pain (Pellegrini et al., 2005), a situation that will also be regulated by endocannabinoid and glucocorticoid receptors (Papini et al., 2015). Similarly, several studies have linked frustration with substance abuse. Research supports that substance abuse occurs in order to reduce the negative emotions produced in the individual after an experience of frustration (Kamenetzky et al., 2009; Torres & Papini, 2016). Similarly, an increase in alcohol consumption was also found when the animals were subjected to stress situations by restriction (Wille-Bille et al., 2017).

A review of the pharmacological interactions with cSNC is presented in Table 2. Results have been restricted to cSNC protocol. To sum it up, *benzodiazepines*, such as CDP, Flurazepam and Midazolam, have eliminated the contrast by the second day. Thus, a greater recovery has been found when these drugs were administrated on the second day, as opposed to the first day. The greatest effects have been found with CDP at 8 mg/kg and CDP administrated with ethanol. Similarly, *ethanol* administration reduces contrast in a dose-dependent manner (from 0.75 g/kg of 15% (w/v) ETOH). However, there is not any dose of alcohol that eliminated the contrast due to a lower dose did not have any effect, and a higher dose (over 2 g/kg of 15% (w/v) ETOH) produced motor incoordination (Becker and Flaherty, 1982).

Little research has been conducted about *barbiturates* in SNC procedure. Sodium amobarbital reduces the contrast, both the first and the second day, but that reduction is very small. Higher doses of sodium amobarbital (over 20 mg/kg) had no effect in contrast (Flaherty et al., 1982).

Due to the correlation with emotional disorders, *serotonergic agents* have also been studied. No results have been found either the first nor the second postshift day. The administration of insulin reduced the consummatory contrast, but it is supposed that the calories which provide the insulin reduce the necessity of sucrose intake produce in contrast (Flaherty et al., 1983). No effect has been found in either *dopaminergic nor noradrenergic agents* administration.

Table 2. Pharmacological interactions in consummatory Successive Negative Contrast. It has been grouped according to the classification of the drug administrated and the effectiveness in the first postshift day and the second postshift day. The dose, the means of administration, and the effectiveness has been determined considering the Postshift Session Day (1 or 2).

Drugs		**Author**	**Negative Contrast Day 1**			**Negative Contrast Day 2**		
			Admin	Doses	Effectiveness	Admin	Doses	Effectiveness
Benzodiazepines	Chlordiazepoxide (CDP)	Vogel & Principi (1971)	IP	8 mg/kg	0	IP	8 mg/kg	+
		Flaherty et al. (1980)	IP – R*	8 mg/kg	+	IP	8 mg/kg	+
			IP		0			
		Flaherty et al. (1986).	IP	8 mg/kg	+ (the 7th minute of access)			
		Flaherty, Grigson & Lind (1990)	IP	5, 10, 15 mg/kg	0	IP	5 or 10 mg/kg	+
		Ortega et al. (2014)	IP (after trial)	5 mg/kg	-	IP	5 mg/kg	+
	Flurazepam	Flaherty et al. (1992)				IP	5, 10, and 20 mg/kg	+
	Midazolam	Becker (1986)				IP	0.25 or 0.50 mg/kg	0
							1.00, 1.25 or 2.00 mg/kg	+
		Flaherty et al. (1990)				IP	1.00	+
Ethanol		Becker & Flaherty (1982)	IP - R	0.75, 1.0 or 2.0 g/kg 15% (w/v) ETOH	0	IP R	0.75, 1.0 or 2.0 g/kg 15% (w/v) ETOH	+

				0.25 or 0.50 g/kg 15% (w/v) ETOH	0		0.25 or 0.50 g/kg 15% (w/v) ETOH	0
Ethanol + CDP		Becker & Flaherty (1983)					CDP 4mg/kg ETOH 0.5 g/kg	+
Barbiturates	Sodium amobarbital	Flaherty & Driscoll (1980)	IP	17.5 mg/kg	+ (small effect)	IP	17.5 mg/kg	+ (small effect)
		Flaherty et al. (1982)	IP	15 or 17.5 mg/kg	+ (small effect)	IP	15 or 17.5 mg/kg	+ (small effect)
				20 mg/kg	-		20 mg/kg	-
Serotonergic agent	Methysergide (Ant)	Becker (1986)				IP	3, 6, 12 mg/kg	0
	Cinanserin (Ant)	Becker (1986)				IP	10 and 15 mg/kg	+
							5 and 20 mg/kg	0
	Ketanserin(Ant)	Flaherty et al. (1990)				IP	2 or 8 mg/kg	0
	Ritanserin(Ant)	Flaherty et al. (1990)	IP	0.63 or 2.5 mg/kg	0	IP	0.63 or 2.5 mg/kg	0
	PCPA (Ant)	Grigson & Flaherty (1991)	IP	150, 300 mg/kg	0	IP	150, 300 mg/kg	0
	Imipramine	Flaherty et al. (1977)	IP R	8 or 16 mg/kg	0	IP R	8 or 16 mg/kg	0
	Gepirone (Ag)	Flaherty et al. (1990)				IP	2.5, 5.0 and 10.0 mg/kg	0
	Buspirone (Ag)	Flaherty et al. (1990)	IP	0.125, 0.25, 0.5, 1.0, 2.0, 15.0 mg/kg	0	IP or SC	0.125, 0.25, 0.5, 1.0, 2.0, 15.0 mg/kg	0

			IP - C	0.5 or 2.0 mg/kg	0	IP - C	0.5 or 2.0 mg/kg	0
	Insulin	Flaherty et al. (1983)	IP	5.4 U/kg	+?			
Dopamine agent	Haloperidol (Ant)	Flaherty et al. (1992)				IP	.1, 0.5, or 1.0 mg/kg	0
	Chlorpromazine (Ant)	Flaherty et al. (1992)	IP	l, 3, or 5 mg/kg	0	IP	l, 3, or 5 mg/kg	0
	Amphetamine (Ag)	Flaherty (1996) (unpublished data)			0			0
Noradrenergic agents	Clonidine (Ag)	Flaherty et al. (1987)				IP	3.12, 6.25, 12.5 25.0 and 50.0 µg/kg	0
Acetylcholine	Scopolamine (Ant)	Becker (1986)				IP	0.25 or 0.50 mg/kg	0
Histamine	Pyrilamine (Ant)	Becker (1986)				IP	3 or 6 mg/kg	0
		Flaherty et al. (1990)	IP	6 or 12 mg/kg	0			
	Cyproheptadine (Ant)	Becker (1986)				IP	3, 6. or 12 mg/kg	+
		Grigson & Flaherty (1991)	IP	3 or 6 mg/kg	+	IP	3 mg/kg	+
							6 or 12 mg/kg	0
Opiates	Morphine (Ag)	Rowan & Flaherty (1987)	IP	2, 4 and 8 mg/kg	+ (small effect)	IP	2, 4 and 8 mg/kg	+ (small effect)
				0.5, 1.0, 16.0 mg/kg	0		0.5, 1.0, 16.0 mg/kg	0
	DPDPE (Ag)	Wood et al. (2005)	IP	24 g/kg at a volume of 1 ml/kg)	+ (small effect)		24 g/kg at a volume of 1 ml/kg)	0

	Naloxone (Ant)	Rowan & Flaherty (1987)	IP	0.50 mg/ kg	- (small effect)
		Pellegrini et al. (2005)	IP	2 mg / kg	-
	Naltrindole (Ant)	Pellegrini et al. (2005)	IP	1 mg / kg	- (small effect)

IP: Intraperitoneal administration; SC: Subcutaneous administration; IP-C: IP chronic; IP-R: IP repeated; IP*: Administration in preshift phase; +: Reduce contrast; - Increase contrast; ?: Controversial result; Ag: Agonist; Ant: Antagonist

Antihistaminic pyrilamine and cyproheptadine have shown controversial results due to the ineffectiveness of pyrilamine and the greater results of cyproheptadine. Cyproheptadine is an antihistaminic with additional anticholinergic, antiserotonergic, and local anesthetic properties. For this reason, this drug is commonly used in the treatment of migraines, allergy and serotoninergic syndrome between other (Grigson & Flaherty, 1991). The unspecified action could be the main reason of its effectiveness in SNC.

Finally, several studies have found that stress activates opiates analgesic mechanisms (Drolet et al., 2001). This activation has also been found in frustration. For example, it was found that the administration of morphine (a non-selective opioid agonist) before the first postshift session reduces the cSNC (Wood et al., 2005) whereas the administration of naloxone (a non-selective opioid antagonist) increases the effect of the cSNC (Daniel et al., 2009).

The global drug effectiveness in cSNC was studied by Flaherty (1996). The author designed a formula for frustration that compare the last preshift day and the first preshift day between the drug and the vehicle. The results for the clearest finding in each drug have been represented in Figure 7.

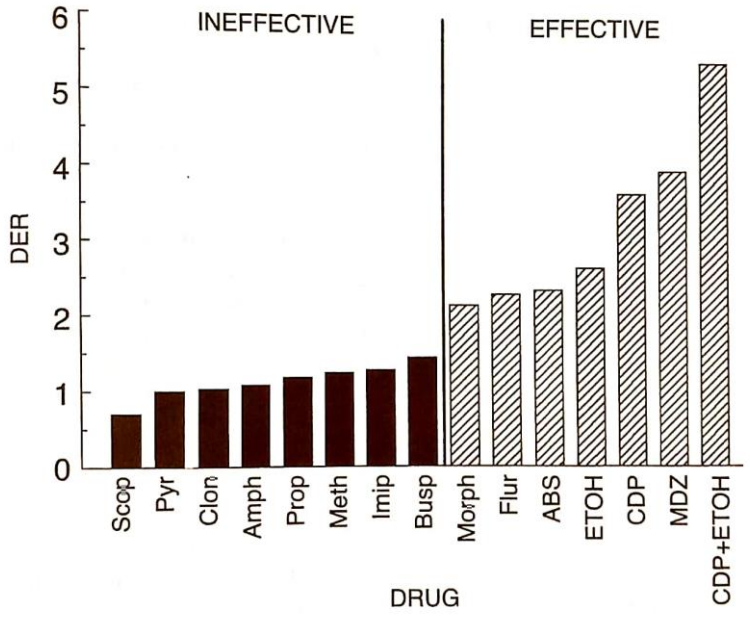

Figure 7. Drug Effectiveness Ratio in Consummatory Successive Negative Contrast. DER determined the effectiveness of the drug from 0 to an final not delimited. Similarly, it has been separated the drug effectiveness considering the behavioral response. A great DER has indicated a great behavioral effectiveness. The effectiveness of the drug in the behavioral results has also been represented. Ineffective drugs are shown with bars filled in black and effective drugs with bars filled in lines (Source: Flaherty, 1996).

Results represented in DER (Figure 7) are specific to the reduction of frustration in the first postshift day but not in the second postshift day. However, the behavioral differences seem to correlate to the DER's results due to the ineffective drugs have a lower DER score and the effective drugs have a higher score in it.

3. Sigma-1 receptor

3.1.General description and structure

The sigma-1 receptor (σ1 receptor or sig1R) has been identified as a molecular chaperone regulated by ligand in the endoplasmic reticulum (ER) of cells that interacts with other proteins to modulate their activity (Alon et al., 2017;Su et al., 2010). More specifically, the Sig1R is an intracellular unique ligand-regulated molecular chaperone composed of 223 amino acids with a high homology between species, which does not have a structural relationship with any other known mammalian protein (Alon et al.,2017).

Initially, Martin et al. (1976) considered the sigma receptors (SigR) as a subclass of opioid receptor due to the psychotomimetic actions observed with the racemic N-allyl-normetazocine ((±)-SKF-10,047) and other analogues. These effects could not be explained by the actions on μ-opioid or κ-opioid receptors and contributed to the proposal of a σ-opioid receptor, derived from the first letter "S" of SKF-10,047. This initial confusion was due to the enantiomeric selectivity of the sigma receptors for the (+)-SKF-10,047 rather than for their (–)-isoforms. The latter is blocked by opioids antagonist whereas the (+)-SKF-10,047 lacks affinity for opioid receptors but interacts with the phencyclidine (PCP) binding sites (Cobos et al., 2008; Maurice and Su et al., 2009).

Therefore, two subtypes of sigma receptors were considered, based on the selectivity profile of some ligands and the molecular mass: Sig1R and sigma-2 receptors (Helewell et al., 1994; Quirion et al., 1992). The Sig1R was first cloned from guinea pig liver (Hanner et al., 1996) and later from other species including mice, rats and human tissues (reviewed by Cobos et al., 2008). By contrast, the sigma-2 receptor was recently identified as a transmembrane protein 97 (TMEM97) related to cholesterol homeostasis (Alon et al., 2017b).

The structure of Sig1R is still under debate, since initially it was considered that it presented only a single transmembrane domain (Hanner et al., 1996) and later other studies have proposed models with two transmembrane domains (Aydar et al., 2002; Ortega-Roldan et al., 2015). However, recent studies have ascertained that Sig1R forms oligomers (e.g., trimers) at the ER membrane, creating a Sig1R ligand–binding pocket (Chu et al., 2013; Schmidt et al., 2016). A crystallographic study demonstrated that Sig1R has a single transmembrane domain in the middle of the protein, supporting the structure proposed initially (reviewed in Hayashi, 2019). Today, the Sig1R is considered to be a non-G-protein coupled, non-ionotropic intracellular chaperone at the ER that modulates Ca^{2+}-signaling (Penke et al., 2018)

3.2.Subcellular and anatomical distribution

Subcellular distribution studies in neurons have shown that the Sig1R is primarily located in the membrane of mitochondria, some cisternae of ER, and the plasma membrane (Alonso et al. 2000; McLean and Weber 1988). They are found at high levels in the interface with mitochondria at the mitochondria-associated ER membrane (MAM) (Su et al., 2010). MAM interacts physically with the mitochondrial outer membrane and plays an important role in the transfer of Ca^{2+} from the reticulum to mitochondria, stimulating oxidative metabolism and regulating Ca^{2+} homeostasis (Pinton et al., 2008). It has been suggested that, on activation, the Sig1R is translocated from the ER to other areas of the cell, such as the plasma membrane or the nuclear membrane (Hayashi and Su 2001; Morin-Surun et al. 1999; Su et al., 2010).

Sig1R are widely distributed in peripheral organs (e.g., heart, kidney, liver, digestive tract or skin) and different areas of the central nervous system (CNS), where they have been studied in detail. They are broadly distributed in the brain, and concentrated in specific areas involved in memory, emotion and sensory and motor functions (e.g. hippocampus: highest density in dentate gyrus; hypothalamus, mesencephalon, olfactory bulb) (reviewed in Cobos et al., 2008; Kulkarni and Dhir, 2009; Maurice and Goguadze, 2017; Merlos et al., 2017). Thus, based on a positron emission tomography (PET) study in the human brain using the Sig1R radioligand [^{11}C]SA4503 has shown that Sig1R are distributed throughout the grey matter of the human brain (Toyohara et al., 2014). Moreover, cellular distribution of Sig1R exhibited

a high distribution in the frontal cortex, hippocampus and striatum, but lower in the cerebellum (Toyohara et al., 2009) (see Figure 8).

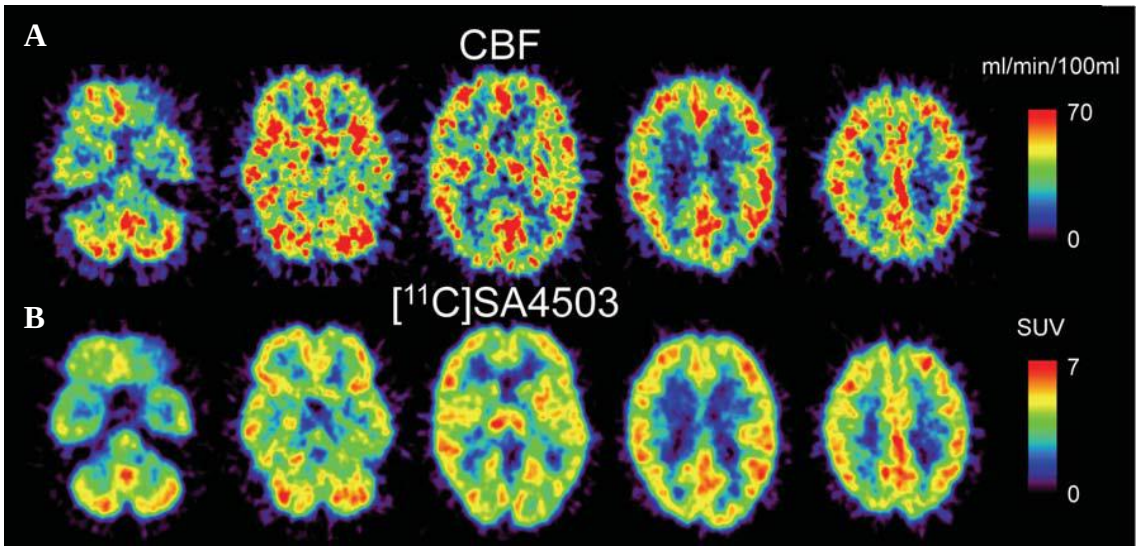

Figure 8. Neuroimaging of sigma 1 receptor activity in repose and after [^{11}C]SA4503 administration. Cerebral blood flow (CBF) and sigma1 receptors in the brain of a healthy human subject. (A) The image represents the CBF using [15O]H2O and calculated as the ml/min/100 ml tissue. (B) The image shown the static images were acquired 40-60 min after the injection of [11C]SA4503. It is expressed as the standardized uptake value (SUV: regional activity divided by administered dose per body weight) (Source: Toyohara et al., 2009).

These findings about the brain distribution of Sig1R are very significant for the main topic of this Doctoral Thesis, since the mechanism involved in frustration have a close relation with many of the areas where Sig1R are located.

3.3.The pharmacology of sigma-1 receptor

In spite of the fact that Sig1R were discovered more than 40 years ago, the endogenous ligand for this receptor has not yet been identified. The function of the Sig-1R can be regulated by synthetic and endogenous ligands. Endogenous ligands of Sig-1R are several neurosteroids (e.g. progesterone, pregnenolone, allopregnanolone or dehydroepiandrosterone) (Cobos et al., 2008; Su et al., 2010) and the natural hallucinogen N,N-dimethyltryptamine (Fontanilla et al., 2009). However, even though all these substances show affinity for Sig-1R, they are not selective for this receptor.

Sig-1R do not bind prototypical opioid drugs including agonists and antagonists, although they were initially characterized as a subtype of opioid receptors (see 1.1 section). Nevertheless, they bind, with high to moderate affinity, to a wide spectrum of known compounds with different pharmacological applications, such as antipsychotics

(e.g. haloperidol), antidepressants (e.g. fluvoxamine), antitussives (e.g. carbetapentane), drugs for the treatment of neurodegenerative disorders (donepezil), and drugs of abuse (cocaine and methamphetamine) among others (Cobos et al., 2008; Su et al., 2010).

Nevertheless, several selective Sig1R ligands that are considered prototypical compounds for these receptors, are available. These include agonists such as (+)-pentazocine and PRE-084, and antagonists such as BD-1063, BD-1047, NE-100 and S1RA (Cobos et al., 2008; Romero et al. 2012), which have been extensively employed to study the Sig-1R function. One of these compounds called S1RA has proved its selectivity for Sig1R on a panel of 170 targets (Romero et al., 2012). This Sig1R antagonist has been extensively evaluated in pre-clinical models of chronic pain and recently in a Phase II clinical trial, showing efficacy in reducing chemotherapy-induced neuropathic pain with a good safety and tolerability profiles (Bruna et al., 2018; Bruna and Velasco, 2018). S1RA has been the first Sig-1R ligand developed with an intended indication for neuropathic pain treatment.

3.4.Actions of sigma-1 receptors in several states or pathologies

Since Sig1R are present in high density in the CNS (and also in peripheral organs) and modulate many actions involved in different pathologies such as depression and anxiety, amnesic and cognitive deficits, psychosis, neurodegenerative diseases, pain and drug abuse, we will focus on those fields that are more related with frustration. Therefore, the actions of Sig1R with regard to pain, mood disorders (anxiety and depression) and memory will be reviewed.

3.4.1. Sigma 1 receptor and anxiety and depression

Due to the fact that mood disorders such as anxiety and depression play a key role in frustration, focus will be placed on those interactions that have been found between Sig1R stress, anxiety and depression

Evidence of anxiolytic and antidepressant activity of Sig1R ligands has been reported and they have been studied as possible pharmacological tools against these mood disorders (see for review Cobos et al., 2008; Fishback et al., 2010).

Sig1R have shown to modulate learning, memory and neuroprotection functions, and all of these functions have an important role in stress and mood disorders (Li &

Murabito 2016; Maurice & Goguadze 2017). In particular, the administration of the Sig1R agonist PRE-084 after stress, reduced the anxiety in a Post-traumatic stress disorder (PTSD) model in rat (Fujimoto et al., 2012; Ji et al., 2016, 2017). Authors reported an improvement in memory which could be due to the neuroprotective effects of Sig1R agonist drugs. It is suggested that an over expression of Sig1R improves the phosphorylation of brain-derived neurotrophic factor (BDNF) and leads the activation pathways involved in the neurogenesis (Takebayashi et al., 2004; Ji et al., 2017). Similarly, Sig1R regulates the synaptic efficiency and the spatial learning performance (Moradpour et al., 2016).

Recent studies have focused on the modulation of cell stress through psychological stress (Hayashi, 2015) (for more information, see Figure 9). It suggests the HPA axis enhances the spread of cytokines (including tumor necrosis factor-α or TNF-α) which are involved in oxidative stress. Specifically, that oxidative stress may be caused by the glutamatergic receptor activation and dopamine auto-oxidation which, also increases reactive oxygen species (ROS) but Sig1R could protect these cells against oxidative stress involved due to ROS and TNF-α accumulation (Zhao et al. 2014).

Therefore, the Sig1R agonists have been shown to reduce the stress in a conditioned fear procedure due to the fact that Sig1R may induce a potentiation of the neural morphogenesis (Noda et al., 2000; Villard et al., 2011). The administration of the Sig1R agonist (+) SKF-10047 reversed the stress in the conditioned fear while the administration of antagonist NE-100 (and not (+) pentazocine and DTG) blocked that effect (Kamei et al. 1997; Matsuno et al.,1996; Urani et al., 2001;Reddy et al., 1998).

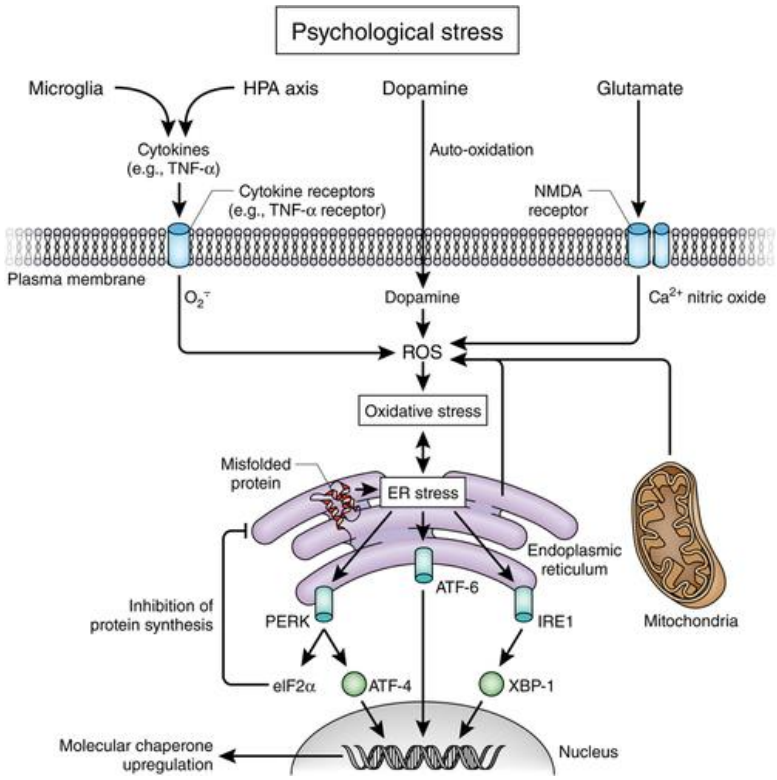

Figure 9. Link between psychological stress and cellular stress. Psychological stress activates the hypothalamus–pituitary–adrenal gland (HPA) axis, in concert with microglia, elevates circulating cytokines, including tumor necrosis factor-α (TNF-α). Cytokines, dopamine auto-oxidation and glutamatergic receptor activation increase reactive oxygen species (ROS), leading to oxidative and endoplasmic reticulum (ER) stress. Dysfunction of mitochondria or ER also increases ROS generation. Accumulation of misfolded proteins, which is caused by ER stress, is sensed by ER stress sensors: protein kinase RNA-like endoplasmic reticulum kinase (PERK), ATF-6 and IRE1. Those sensor proteins collaborate to upregulate molecular chaperones and suppress global protein synthesis to suppress the accumulation of misfolded proteins in the ER (source: Hayashi, 2015).

Some anxiolytics and antidepressant drugs such as imipramine and fluoxetine have shown a high affinity for Sig1R (Tottori et al., 2001). However, one of the most notorious results were found with opipramol which induced a reduction of depression and anxiety in both human and animal models (Müller et al., 2004). This compound acts as a Sig1R agonist with high affinity for this receptor and shows a weak dopaminergic and serotoninergic antagonism. Igmesine, another Sig1R agonist, reduced the colonic spike bursts induced by conditioned fear stress or corticotropin releasing hormone (Gue et al., 1992). Ago and coworkers (2017) suggests that some antidepressant effects of the prototypical antidepressant drugs such as fluvoxamine, could be due to the enhancing of the prefrontal dopaminergic neurotransmission via both 5-HT reuptake inhibition and Sig1R receptor activation under the circulating neuroactive steroid-deficient conditions (Ago et al., 2011, 2017). In addition, studies with Sig1R receptor knockout mice further support the role of these proteins in depression as these mice present a depression-like response in the forced swimming test (FST) (Sabino et al., 2009).

A total of 2.408.700 people in Spain fulfilled the diagnostic criteria of Mayor Depressive Disorder (MDD) in 2020 (WHO, 2020). Both clinical and preclinical trials have been tested to determine the efficacy of sig1R in major depression. As with anxiety models, FST has been used to induce depression in animals (Matsuno et al. 1996a). Igmesine, as well SA4503 (both agonist sig1R), have showed an antidepressant effect while antagonist BD1047 and NE-100 have blocked it (Matsuno et al., 1996a,b). Interestingly, the antidepressant effect of agonist SA4503 appeared after a single dose administration (Matsuno et al., 1996b). Clinical trials have also show that a monotherapy of agonist fluvoxamine improves the psychotic major depression (Shirayama & Hashimoto, 2010).

3.4.2. Sigma 1 receptor and learning and memory impairment

The learning and memory processes are closely linked to the frustration evoked by the reward loss situations (Amsel, 1992). According to the sequential theory, frustration is a cognitive process regulated by the memory (Capaldi, 1966). This theory was supported by Glueck et al. (2015) who found a high expression of pCREB in the prelimbic cortex, the anterior cingulate and the dorsal-medial striatum. These results suggest that the memory consolidation has an important role in the reward devaluation.

Sig1R has been found to modulate memory and learning processes when a state of pharmacological or pathological imbalance is induced. This means that Sig1R receptors are not involved in normal memory functions (Cobos et al., 2008; Maurice and Goguadze, 2017). Likewise, a reduction of sig1 expression have been found in early Alzheimer Disease (AD) case reports (Mishina et al., 2008). This has been confirmed in several pharmacological and pathological models of learning and memory impairment in rodents (Maurice, 2016; Yabuki et al., 2015).

In addition, Sig1R agonists are promising symptomatic drugs in rodent models of cognitive alterations related to pathological aging and neurodegenerative diseases (Fujimoto et al., 2012; Mishina et al., 2008). Several Sig1R agonists such as Igmesine and PRE-084 improved learning ability (Maurice et al. 1996). These compounds also alleviated the memory deficits induced by amyloid toxicity in pharmacological models of Alzheimer's disease (AD). All agonists of the Sig1R mitigated the AD-induced learning impairments in spatial or nonspatial tasks involving short-term as well as long-term memory. These actions were blocked by Sig1R antagonists (BD-1047 and

haloperidol among others) but the antagonists alone did not alter the behavior (positively or negatively) in these models (Maurice and Goguadze, 2017).

3.4.3. Sigma 1 receptor and pain

The role of Sig1R in pain is supported by a vast number of studies (for a review see Merlos et al., 2017; Romero et al., 2016). It has been suggested a potential therapeutic use of Sig1R antagonists for the management of neuropathic pain and other pain conditions including inflammatory, visceral, ischemic, postoperative, and orofacial pain. Their actions in pain can be divided in the modulation of opioid analgesia and their effects per se in different pain conditions.

The first evidences for a role of Sig1R in pain were described by Chien and Pasternak in the 90s. They found that the Sig1R agonist (+)-pentazocine blocked the morphine antinociception whereas the administration of Sig1R antagonists such as haloperidol increased morphine antinociception (Chien & Pasternak 1993, 1994). Additional experiments using other opioids and Sig1R antagonists confirmed the role of these receptors as modulators of opioid analgesia (reviewed by Sánchez-Fernández et al., 2017). Although the effect of sig1R was initially considered to be primarily centrally mediated (Mei & Pasternak, 2002), recent evidences have demonstrated that Sig1R also control peripheral opioid antinociception (Sánchez-Fernández et al., 2013; 2014). In contrast to the potentiation of the opioid analgesia, neither Sig1R antagonism nor Sig1R knockout enhanced the opioid-induced side effects such as constipation, mydriasis, hypermotility or physical dependence (Sánchez-Fernández et al., 2013 and 2014; Vidal-Torres, 2013). Therefore, the association of Sig1R antagonists with opioid agonists has been proposed as a potential strategy to increase the therapeutic window of conventional opioids.

On the other hand, there is broad evidence supporting the role of sig1R in pain perception in the absence of opioid drugs. The first evidence on the role of sig1R in pain control per se was obtained by our group in a formalin-induced pain model in mice. We found that Sig1R knockout mice showed less pain after intraplantar administration of formalin (Cendán et al., 2005a) and that Sig1R antagonists (haloperidol and some of its metabolites) produced dose-dependent analgesia in this pain model, showing a good correlation between their affinity for the sig1R receptor and their analgesic potency (Cendán et al., 2005b). In addition, we found that the Sig1R antagonists BD-1063, BD-

1047, NE-100 and haloperidol antagonized mechanical allodynia induced by intraplantar capsaicin administration in the mouse (Entrena et al., 2009 a,b). These antinociceptive effects on formalin and capsaicin were not blocked by the opioid antagonist naloxone (Cendán et al.,2005; Entrena et al.,2009; Vidal-Torres et al., 2014), indicating that the Sig1R actions are not due to the modulation of the opioidergic system.

Furthermore, there has been reported that sigma-1 inhibition is also effective on the neuropathic pain. In the studies on mechanically-induced sciatic nerve injury, the systemic administration of the Sig1R antagonist S1RA fully reversed the hypersensitivity to mechanical and thermal painful stimuli (Bura et al., 2013; Díaz et al., 2012; Romero et al., 2012). In addition, systemic treatment with selective Sig1R antagonists (BD-1063 and S1RA) was shown to ameliorate neuropathic pain induced by the antineoplastic paclitaxel (Nieto et al., 2012; 2014). The role of Sig1R on inflammatory pain also has been reported. It was found that the systemic administration of several Sig1R antagonists, including S1RA and BD 1063, were able to ameliorate acute inflammatory pain hypersensitivity (Gris et al., 2014; Parenti et al., 2014a; 2014b; Tejada et al., 2014).

Finally, antinociceptive efficacy against visceral pain has been also demonstrated by Sig1R antagonism. In particular, the subcutaneous administration of the Sig1R antagonists BD-1063, S1RA and NE-100, reduced the number of behavioral responses and reversed the referred hyperalgesia in the intracolonic capsaicin-induced visceral pain (González-Cano et al., 2013). Therefore, the pharmacological blockade of Sig1R might provide a therapeutic approach to visceral pain treatment.

Table 3. Pharmacological interactions of sigma ligands. It has been grouped according the objective treatment (depression, PTSD and memory). Similarly, it has been determined the experimental model to induce the objective of the treatment and the test to evaluate it. It has determined the drug and its action in Sig1R, the dose and route of administration and the effectiveness. The animal model has also been indicated.

Treatment	Experimental model	Author	Test	Animal model	Drug	Sig1R	Dose/route	Effectiveness
Depression	Forced swim test	Urani et al. (2001)		Swiss OF1 mice	Igmesine	Ag.	volume of 100 µl/20 g of body weight i.p.	+ (blocked with BD-1047)
					DHEAS	Ag.		+ (blocked with BD-1047)
					(+)-SKF-10,047	Ag.		+ (blocked with BD-1047)
		Matsuno et al. (1996)		ddY mice	SA4503	Ag.	0.1 and 1.0 mg/kg i.p.	+ (reversed with NE-100)
					(+)-pentazocine	Ag.	5.0 mg/kg i.p.	+(reversed with NE-100)
					DTG	Non-specific ag	5.0 mg/kg i.p.	+ (reversed with NE-100)
					Igmesine	Non-specific ag.	10.0 mg/kg i.p.	+
		Reddy et al. (1998)	Porsolt forced swim test	Laka mice and Wistar rats	DHEAS	Ag.	5, 10 and 20 mg/kg, s.c.	+ (blocked with NE-100)
					PS	Ag.	5, 10 and 20 mg/kg, s.c	+ (blocked with NE-100)
PTSD	Combined stress paradigm	Ji et al. (2016)	Open Fied, Elevated plus maze and Morris water maze	Sprague-Dawley rats	PRE-084	Ag.	0.6 mg/kg for 7 consecutive days	+
Memory	Scopolamine-	Zvejniece	Passive avoidance (PA)	ICR and Balb/c	E1R	Ag.	0.1, 1 and	+

	induced cognitive impairment	et al., 2014	and Y-maze tests	mice and Wistar rat			10 mg·kg^{-1} i.p.	
	β_{25-35}-amyloid peptide-induced amnesia	Maurice et al., 1998	Spontaneous alternation performances and Step-down type passive avoidance test	Swiss mice	PRE-084	Ag.	0.3, 1 and 3 mg/kg s.c.	+ (blocked by haloperidol or BMY-14 802)
					SA4503	Ag	0.3 mg/kg s.c.	+
							0.1 and 1 mg/kg sc	0
					(+)pentazocine	Ag.	0.1, 0.3 mg/kg s.c.	+
							0.3 mg/kg s.c.	0

Rationale, hypothesis and goals

1. Rationale

Nowadays, people must live in a complex society in which the presence of stress disorders, such as anxiety or depression, is becoming more frequent. A recent study has determined that 9 out of 10 Spanish citizens have suffered stress in the last year (Maset, 2017). This stress could be expressed as frustration when is due to a downshift of reinforcements. However, frustration is not the same in two people. A childhood trauma, the personality, the death of a relative, a divorce or economic concerns are just a few examples of situations which could trigger an anxiety disorder (Kamenetzky et al., 2009). The study of parameters that enhance or inhibit the appearance of the anxiety disorders is still unknown. Personal traits or the circumstances that involves frustration could influence in the final outcome (Sanchez et al., 2001). Specifically, it is suggested that a previous experience with the lower reinforcer could reduce the negative effect produced by the reward devaluation (Flaherty et al., 1983; Shackman et al., 2011).

Currently, the anxiety drugs commercialized could not meet the expectations of their consumers. The pathogenesis of these stress-related diseases has not been fully clarified, but anxiolytics and antidepressants are used primarily for its treatment (Tottori et al., 2001). These treatments need, at least, a time of two weeks to have measurable results and, most of them, have a great number of side effects whose drug resistance, tolerance or other secondary effects when used continuously are extremely worrying. A possible development of an abuse disorder could provoke serious consequences for the consumer (Hayashi, 2015).

The integration of new drugs that act on sigma ligands has been suggested for the treatment of these mood-related disorders (Fishback et al., 2010; Li & Murabito 2016; Maurice & Goguadze 2017; Tottori et al., 2001). Therefore, it has been desired the development of a therapeutic drug with a new mechanism of action (Cobos et al., 2008;). Recent publications have indicated that Sig1R act as a regulator in the central nervous system and, likewise, it has physiological functions such as learning, memory and neuroprotection (Li & Murabito 2016; Maurice & Goguadze 2017. All these facts have been suggested as important point to consider in stress disorders (Turk et al., 2011).

The neurobiological bases on which anxiolytic drugs exert their actions are not completely known. It is suggested that antidepressants cause a modulation in the

process involved in these disorders but, it is unknown the exact action or at what point in the cascade of events a change is provoked (Gue et al., 1992).

On the other hand, the influence of opiates drugs has suggested a common modulation between anxiety process derivates of frustration and pain (Papini et al., 2015). Neuroimaging results have supported that theory due to a cerebral activation in a shared area to pain and negative effects, the rostral cingulate cortex (Shackman et al., 2011). The activation of the cingulate cortex is highly involved in stress because the emotional situations can hurt as a physical one. Thus, when there is a stressful event, the body cannot feel a physical blow or, on the other hand, when there is a chronic pain, the person may differ psychological discomfort (Papini et al., 2015).

The related research to stress disorders in animal models has been proposed as little translational or clinical practical (Kameneztky et al., 2009). Therefore, SNC has been suggested as a very useful animal model in which the expectations or interpretations of the animal itself are the cause of frustration. Likewise, the SNC has shown a reduction of its frustrating consequences when an anxiolytic has applied (Flaherty, 1980; Ortega et al., 2013). Thus, the test on SNC of drugs that act on Sig1R could open up the possibility of new targets which could be very useful in clinical setting.

2. Hypothesis and goals

The main goal of this Doctoral Thesis has been to study the frustration process induced by the paradigm of devaluation of the reward called Successive Negative Contrast (SNC). To study this process, behavioral, physiological and pharmacological approaches has been used.

Based on the sensitivity of the frustration to prior experiences to the devaluation of the reinforcement (Flaherty et al., 1983), it has been presumed that the experimental manipulation in the preshift phase of the SNC causes different behavioral responses associated with that previous experience. Flaherty et al. (1983) find a mitigated expression of frustration after a gradual increase of the reinforcer in the consummatory SNC procedure. Neurobiological differences between the consummatory and instrumental procedures suggest that the response between these two types of CSN may be different (Flaherty, 1996; Pellegrini & Papini, 2007).

Therefore, it is also hypothesized that the group with a previous experience of high reinforcer will present greater frustration when its reinforcement is devalued compared to the control group, which will always receive the low reinforcement. Similarly, it is hypothesized that the level of frustration will be higher in the experimental group compared to the increasing group that will receive an in-crescendo reinforcer during the preshift phase in the instrumental SNC procedure **(Hypothesis 1**).

To test this hypothesis, the *first goal* has been to assess the behavioral differences derivates to the manipulation of expectations in the devaluation of rewards in the iSNC paradigm. For this reason, 3 groups have been carried out: a control group, an experimental group and a rising group. The experimental group has had access to 12 pellets during the preshift sessions, while the control group has 2 pellets. The rising group had a gradual increase during the sessions (2, 4, 6, 8, 10 and 12 pellets). Subsequently, all animals had access to 2 pellets in the postshift phase. In order to record behavioral differences associated with experimental manipulation, the time took for animals to go through the maze to reach the expected reinforcer was recorded.

Likewise, previous studies have indicated the existence of a neurobiological and pharmacological correlation between physical pain and stress or anxiety processes (Torres et al., 2015). It is suggested that an anxiogenic response induce by a reward devaluation can modulate the physical pain (Mustaca and Papini, 2005). In that sense, it is expected that animals that had previous experience of frustration presented a greater hypoalgesic response, that is, a decrease in sensitivity and an increase in the threshold to painful stimuli in the face of such frustration compared to animals that were not frustrated (**Hypothesis 2**).

To test this hypothesis, the *second goal* has been to study differences in the perception of pain (measured by the von Frey test and the Hargreaves test) after a previous experience of frustration induced by the cSNC. On the other hand, the *third goal* has been to study the behavioral responses referred to the evaluation of such perception. This it was carried out during the first and second day of the postshift phase (that is, the first and second day of devaluation) immediately after and 300 minutes later the devaluation.

Finally, previous pharmacological evidence pointed out Sig1R as a modulator of processes associated with frustration experiences. The Sig1R antagonism has shown to

be effective against pain (Zamanillo et al., 2013; Merlos et al., 2017), whereas the Sig1R agonists have demonstrated an anxiolytic effect and an improvement in learning and memory (Urani et al., 2001; Maurice and Goguadze, 2017). That is the reason why it is hypothesized that Sig1R could modulate processes associated with frustration (**Hypothesis 3**).

Our *fourth goal* was to evaluate the effects of Sig1R ligands on frustration. To achieve this goal, we administrated the selective Sig1R antagonists BD-1064 and S1RA and the selective Sig1R agonists PRE-084 and igmesine in the first postshift day of the cSNC model. The Sig1R antagonists could be expected to act reducing the pain derived from the frustration process whereas the Sig1R agonists might mitigate the frustration-induced anxiety.

Material and methods

1. Experimental animals

The subjects were male Wistar rats purchased from Harlan Laboratories (Barcelona, Spain), Envigo Laboratories (Barcelona, Spain) and Charles Rivers (Les Oncins, France). Rats were approximately 75 – 90 days old at the beginning of the experiment.

Rats were housed individually in polycarbonate cages with ad lib water, in a room with constant temperature (24 ◦C) and humidity (50-60%). Animals were housed under a 12:12 h cycle of light: darkness (lights on at 08:00 h) and food deprived to 82-85% of their ad lib weights throughout the experiment.

The experimental protocol was approved by the University of Granada Research Ethics Committee (20-08-15-294 and 09/08/2019/134).

2. Animal models of frustration

The animal model carried out to induce frustration has been the Successive Negative Contrast. SNC procedure compares an experimental group with a control group. The experimental group receives a previous experience of high reinforcer during a preshift phase, which is devalued during the postshift phase to a reinforcer of lower quantity and/or quality. On the other hand, the control group always receives the lower reinforcer. Thus, during the present Doctoral Thesis the control group is mentioned as a group that always receives a low reinforcer and the experimental group as a group that receives a devaluation of the reinforcer.

There are two modalities for the SNC procedures: instrumental and consummatory, as it will be described below.

2.1. Instrumental Successive Negative Contrast

The instrumental successive negative contrast (iSNC) procedure has been used in Experiment 1. iSNC requires of an instrument that includes a task to learn. The instrument consisted of a straight maze of dimensions 120 x 14 x 11 cm with an exit area of 20cm, a goal area of 20cm and a central area of 80cm. Similarly, pellets “Bio

Serv. Dustless Precision Pellets" were used during the experiment. The dependent variable recorded in this protocol is the time in traveling the maze.

In the iSNC task, animals have to run across a lineal maze for receiving little balls of sugar called *pellets*. The high-value sucrose amount (usually 12 *pellets*) has been administrated in 6 trials every day for 10 days, followed by 4 sessions with 6 trials every day of access to a low-value sucrose amount (usually 2 *pellets*). Performance of downshifted animals is compared to the behavior of animals that have always received access to 2 *pellets* (unshifted controls). The iSNC effect involves a suppression of running behavior after the 12-to-2 *pellets* downshift followed by a recovery to the level of unshifted controls. The initial suppression (typically observed during the first devaluation session) and the recovery that follows (normally starting during the second devaluation session) are dissociable stages of the iSNC effect (Flaherty, 1996).

Table 4. Experimental design in Experiment 1. Animals were grouped according to the experimental situation (shifted, unshifted and rising group). It has been indicated the days and the quantity of food (pellets) during the preshift and postshift phase

Phase	Day	Control Group (n=8)	Experimental Group (n=8)	Rising Group (n=8)
Preshift	1	2 pellets	12 pellets	2 pellets
	2			4 pellets
	3			6 pellets
	4			8 pellets
	5			10 pellets
	6			12 pellets
Postshift	7	2 pellets		
	8			
	9			
	10			

The procedure used in Experiment 1 was similar to the one previously described by Flaherty et al. (1983). A brief summary of this experiment has been represented in Table 4. Animals were habituated for 3 days. Subsequently, they spent 6 days for the preshift

phase and 4 days for the postshift phase. Twenty-eight rats were grouped into 3 groups: 1 control and 2 experimental. Nine of these rats were part of the control group, so they received 2 pellets in both phases (group 2-2). Another 9 rats were part of the experimental group of gradual increase. In this case these rats received a gradual increase of 2 pellets each day during the preshift phase and 2 pellets in the postshift phase (group 2, 4, 6, 8, 10, 12-2). The last 9 rats were part of the second experimental group. These rats received a constant reinforcement of 12 pellets during the preshift phase and 2 *pellets* in the postshift phase (group 12-2).

Pretraining

On the first day of the habituation phase, 5 trials were carried out with each rat independently of the group to which it belonged (there was about 20 minutes between trial and trial). Each trial consisted of 1 minute of free travel through the maze without *pellets*. On the second day of habituation, a total of five trials were carried out, which could be of three types: The first type included two tests of 1-minute duration each in which the rat traveled freely through the labyrinth without pellets. After the first type of test, the second type was performed, where the rats were left in the goal zone with 12 pellets (experimental group) or with 2 pellets (rising and control group). The maximum duration time was 1 minute. After the second type, a last test was carried out in which the pellets (12 or 2) were placed along the labyrinth and the rat exposed to it for 1 minute. On the last day of habituation, 3 tests were carried out with each rat in which the rats were left locked in the goal zone with 12 or 2 pellets depending on the experimental group to which they belonged. If the rat did not eat the pellets during this period, they were left for 30 seconds in the goal zone and then were taken it out regardless of whether they had eaten them or not.

Preshift

Preshift trials lasted 6 days. In this phase, 6 trials were carried out per day with approximately 20 minutes between them. First, each trial began with the placement of each rat in the exit box with the door closed. After the door was opened, the rat was allowed to go through the maze for a maximum of 20 seconds. However, if the 20 seconds have passed without reaching the goal, the rat was gently pushed to reach it and the maximum time (20 seconds) was registered. When it arrived at the goal zone, the door was closed, and a maximum period of 30 seconds was allowed for the

consumption of pellets. The number of pellets in the goal box depended on the experimental group assigned to each rat. There were 2 pellets for group 2-2, for group 12-2 there were 12 pellets, whereas for group 2,4,6,8,10,12-2 the number of pellets for the first day of preshift was 2, increasing by two pellets each day until the last day with a total of 12 pellets. Finally, the rat was removed, and the labyrinth cleaned when necessary.

Postshift

To finalize, we carried out the postshift phase for 4 days. This experimental condition had 6 trials per day with approximately 20 minutes between each trial. The procedure performed was the same as in the preshift phase, but in this case, all the subjects received 2 pellets in the goal box, independently of the assigned group.

2.2. Consummatory Successive Negative Contrast

The consummatory successive negative contrast (cSNC) procedure was performed in a 15 cm x 30 cm x 30 cm plexiglass box. Sucrose consumption was recorded through burettes graduated at 0.01 ml in Experiment 2 and through grams per kilo in Experiment 3. Results found in Experiment 3 have considered the density of the sucrose concentrations (4% and 32%) for gr/kg calculi.

Sucrose was prepared per grams. Thus, 32% sucrose requires 32 grams of commercial sugar per 68 grams of distilled water. On the other hand, 4% sucrose requires 4 grams of commercial sugar per 96 grams of distilled water.

Preshift

This phase implies unexpected variations in reward magnitude or quality. SNC implies not the elimination, but a devaluation of the magnitude or quality of the reward. In the cSNC task, , animals receive free access for 5 min to a high-value sucrose solution (32% sucrose) during several daily sessions (shifted group), compared to the consummatory behavior of animals that always receiving access to 4% sucrose (unshifted controls).

Postshift

During postshift phase, all rats had access to 4% sucrose for several sessions independently of the group they belong to.

The cSNC effect involves a suppression of consummatory behavior after the 32-to-4% sucrose downshift followed by a recovery to the level of unshifted controls. The initial suppression (typically observed during the first devaluation session) and the recovery that follows (normally starting during the second devaluation session) are dissociable stages of the cSNC effect (Flaherty, 1996). The second experiment lasted for 19 days (16 days of preshift phase and 3 days of postshift phase). On the other hand, the third experiment lasted for 15 days (10 days of preshift phase and 5 days of postshift day).

Drug Effectiveness Ratio

Flaherty (1996) determined a formula specifically designed for the Consummatory Successive Negative Contrast (Figure 10). The original formula designed by Flaherty considered lick frequency due to the initial measurement in the procedure. However, it has been used to grams per kilo in the Experiment 3 which has studied the implications of sigma ligands in Consummatory Successive Negative Contrast.

$$DER = \frac{[P(unshiftedvehicle)] - [P(shiftedvehicle)]}{[P(unshifteddrug)] - [P(shifteddrug)]}$$

Figure 10. Formula for calculating the Drug Effectiveness Ratio. The DER formula discounts the preshift differences in lick frequency by examining postshift data in proportion of consumption (P). Proportion is measured by frequency of the last preshift day divided by the frequency of the first postshift day plus the last preshift day.

The formula showed in Figure 10 tried to compare the proportion of consumption of the shifted group compare to the unshifted group during the last preshift day and the first postshift day. Thus, this formula indicated how has been reduced the consumption in the shifted group compare to the unshifted group. Consequently, it represents the efficacy in comparison with the vehicle circumstances, that is, with the administration of saline. A great reduction indicates a low efficacy and, similarly, a low punctuation close to 0. On the other hand, a low reduction indicates a great drug effectiveness.

3. Animal models to assess pain sensitivity

3.1. Von Frey test

Mechanical hypoalgesia was tested with the von Frey Test (Chaplan et al., 1994). The von Frey filaments (Touch-Test Sensory Evaluators) provided by North Coast Medical Inc. is a non-invasive evaluation test of the levels of skin sensations throughout the body with objective and replicable results. Each filament is individually calibrated to offer its specific strength within a standard deviation of 5%. The rats were evaluated on the right or left hind legs, being half of them on the right leg and the other half on the left. The up and down method was used to determine the pain response threshold.

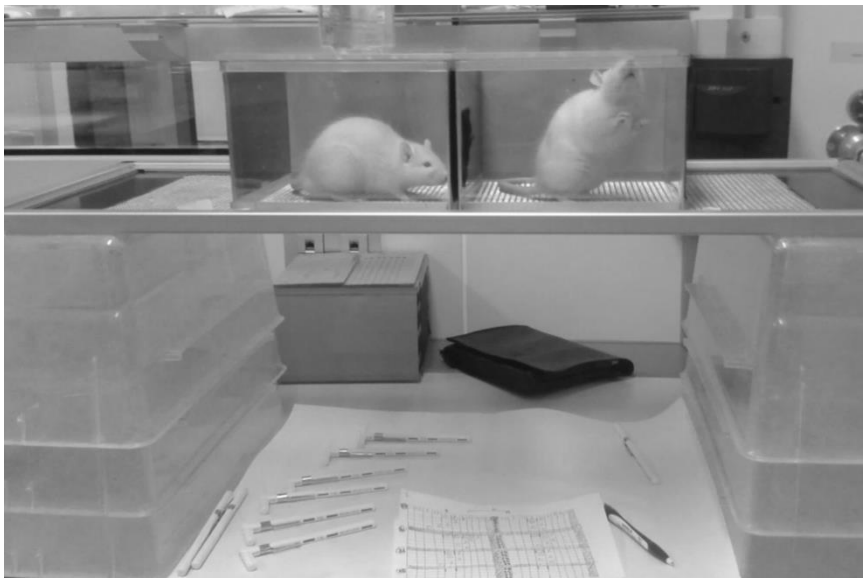

Figure 11. Procedure of von Frey Test. The von Frey filaments are exposed above the photograph. In the moment of the photo the rats were being habituated. Animals were separated by a black Plexiglas panel. The floor where the animals were situated was a metal grid which allowed the evaluation with the filaments.

Before the cSNC protocol, animal was tested for a baseline previous to any experimental manipulation. Von Frey testing occurred 5 and 300 min after the first and the second downshift sessions. Half of the animals were tested the first day and the other half during the second day. To minimize novelty effects with the evaluation boxes, animals were familiarized before the critical test sessions according to the following schedule. Two days before the start of cSNC training and 8 days after preshift sessions (every other day), rats were exposed to the von Frey boxes for 5 min. These preexposure sessions occurred 5 min after preshift contrast sessions and no assessments were done with von Frey filaments.

In every test, each filament was applied three times for 2-3 s, separated by 5-s intervals using the up-down paradigm (Chaplan et al., 1994). Testing started with the 2-g (19.6 mN) von Frey filament (i.e., the middle of the range). The filament was manually pressed against the paw's plantar surface with sufficient force to cause a depression in the skin. The paw chosen for stimulation was always the same for a given animal and it was counterbalanced for right and left hind paw within each group. In each consecutive test, if there was no response to the filament, a stronger stimulus was then selected; if there was a positive response, a weaker one was then used. The response to the filament was considered positive when immediate withdrawal or shaking of the paw was observed. Observers were blind with respect to the contrast assignment of the subject (i.e., 32% vs. 4%).

The statistical analysis in von Frey test, pain sensitivity was expressed as a mechanical threshold producing a response in 50% of the trials. This paw withdrawal threshold value was calculated using the following formula (Figure 12):

$$50\%\ \text{threshold (g)} = 10^{(Xf + \kappa\delta)} / 10{,}000$$

Figure 12. Formula for pain withdrawal value. Xf is the value (in log units) of the final von Frey filament used, κ is the tabular value for the pattern of positive/negative responses, and δ is the mean difference (in log units) between stimuli (Source: Chaplan et al., 1994).

3.2. Hargreaves test

Heat hypoalgesia was tested with the Hargreaves test, also called Plantar test (Hargreaves et al., 1988) with slight modifications, as previously reported (Nieto et al., 2008). The Hargreaves test measures the response to infrared heat stimuli applied to the plantar surface. The heat source is focused below the surface of the plant and the paw withdrawal latency and the infrared intensity are recorded. It allows the measurement of the acute nociceptive thermal threshold in laboratory animals.

Before the cSNC protocol, the animals were tested for a baseline previous any experimental manipulation. The habituation was done in individual opaque evaluation chambers (Hargreaves boxes) placed on a glass floor at 30 °C. The chambers boxes were the same as used in von Frey procedure.

Two days before the start of cSNC training and 8 days after preshift sessions (every other day), rats were exposed to the Hargreaves boxes for 5 min. These preexposure sessions occurred 5 min after preshift contrast sessions. After 5 min of habituation, a beam of radiant heat was focused to the plantar surface of the right hind paw with a plantar test apparatus (Ugo Basile, Comerio, Italy) until a withdrawal response occurred, and the latency to withdrawal response was recorded. The latency of the withdrawal response (as an indirect measure of the heat-pain threshold) was thus recorded automatically. Intensity of the light was adjusted at the start of the experiments to 85 mW/cm2 with an I.R. Heat-Flux Radiometer (Model 37300, Ugo-Basile), and this intensity was not changed throughout the experiments. Radiant heat (42–43°C through the glass floor, for a maximum of 13 s) was applied. Each rat was tested three times, and a cut-off latency time of 20 s was used in each measurement. Temperature was kept constant throughout the experiment.

Table 5. Experimental design in Experiment 2. Animals were grouped according to the experimental situation (shifted or unshifted), the pain perception test (von Frey or Hargreaves) and the day of evaluation (postshift 1 or 2).

SNC group	Pain perception	Day of evaluation of pain
Unshifted	Von Frey Test	Postshift 1 (n=10)
		Postshift 2 (n=10)
Shifted		Postshift 1 (n=10)
		Postshift 2 (n=11)
Unshifted	Hargreaves	Postshift 1 (n=10)
		Postshift 2 (n=10)
Shifted		Postshift 1 (n=10)
		Postshift 2 (n=10)

The critical measurements of pain sensitivity were recorded for each animal after the first and the second downshift sessions. As in von Frey specifications, testing occurred 5 and 300 min after the first and the second downshift sessions and half of the animals were tested each day. After some annotations of the rat behavior during the von Frey test, plantar tests data were recorded to determinate a possible difference in the rat behavior associated to the contrast experience.

The experimental design in Experiment 2 has been represented in Table 5. Eight groups have been defined depending on the SNC group, the instrument of pain perception and the day of that evaluation.

4. Drugs and drug administration

The drug administration was only carried out in Experiment 3. All drugs were dissolved in sterile physiologic saline (0.9% NaCl). The S1gR ligands were alkalized to an optimum pH with NaOH. All solutions were prepared immediately before starting the experiments and injected SC (volume of 5 ml/kg) in the interscapular area 30 minutes before the procedure in the third experiment. Three days before the administration, the animals were habituated to the handing exposed during the first postshift day.

The Sig1R receptor antagonists used were BD-1063 (1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine dihydrochloride; Tocris Cookson Ltd., Bristol, United Kingdom) and S1RA (4-[2-[[5-methyl-1-(2-naphthalenyl)1H-pyrazol-3-yl] oxy] ethyl] morpholine hydrochloride; kindly supplied by Laboratorios Esteve, Barcelona, Spain). The Sig1R agonists used were PRE-084 (2-[4-morpholinethyl]1-phenylcyclohexanecarboxylate hydrochloride; Tocris Cookson) and Igmesine [(+)-cinnamyl-1-phenyl-1-N-methyl-N-cyclopropylene]; kindly supplied by Laboratorios Esteve, Barcelona, Spain). All the Sig1R ligands that were used, i.e., antagonists and agonists, are considered to be selective for sigma-1 receptors (Cobos et al., 2008; Romero et al., 2016).

The experimental design in Experiment 3 has been represented in Table 6. Twenty-six groups have been defined depending on the SNC group, the drug and the dose administrated.

Table 6. Experimental design in Experiment 3. Animals was grouped according experimental situation (shifted or unshifted) the drug administrated (vehicle, S1RA, BD1063, Igmesine and PRE-084), the action in Sig1R (agonist or antagonist) and the dose (mg/kg).

Agonist/Antagonist	Drug	Doses (mg/kg)	SNC group
-	Vehicle	-	Unshifted (n=8)
			Shifted (n=8)
Antagonist	S1RA	64	Unshifted (n=8)
			Shifted (n=9)
		32	Unshifted (n=8)
			Shifted (n=8)
		16	Unshifted (n=7)
			Shifted (n=7)
	BD1063	16	Unshifted (n=8)
			Shifted (n=8)
		4	Unshifted (n=7)
			Shifted (n=8)
		1	Unshifted (n=7)
			Shifted (n=8)
Agonist	Igmesine	16	Unshifted (n=7)
			Shifted (n=7)
		4	Unshifted (n=8)
			Shifted (n=9)
		1	Unshifted (n=7)
			Shifted (n=7)
	PRE-084	8	Unshifted (n=8)
			Shifted (n=32)
		4	Unshifted (n=7)
			Shifted (n=9)
		2	Unshifted (n=8)
			Shifted (n=7)

For the convenience of the reader, a brief summary of all the experiments has been included (Table 7).

Table 7. Brief summary of main aspect of protocols for Experiment 1, 2 and 3. It has been determined the paradigm used and the days required to pretraining, preshift and postshift phases. Similarly, it has been specified the variable entered in the protocol and if it has been required a specific instrument for this variable. The day of evaluation or administration of the drug has been also included.

Experiment	Paradigm	Pretraining	Preshift	Postshift	Variable entered in the protocol	Instrument entered in the protocol	Day of evaluation	Day of drug administration
Experiment 1	iSNC	3 days	6 days	4 days	Other experimental group with a sucrose rising during preshift	Not applicable	Not applicable	Not applicable
Experiment 2	cSNC	Not applicable	16 days	3 days	Pain perception	Von Frey / Hargreaves	Postshift 1 and 2 (immediately and 300 min after postshift)	Not applicable
Experiment 3	cSNC	Not applicable	15 days	5 days	Sig1R agonist and antagonist drugs	Not applicable	Not applicable	Postshift 1 (30 min before protocol)

5. Data Analysis

Statistical analysis was performed using the SPSS statistical program version 20.0. The homogeneity and the homocedasticity test of the sample were performed prior to any analysis in all cases.

For the analyzes between groups, and in cases of violation of the normality and homoscedasticity of the sample, non-parametric tests were carried out to study differences between more than 2 independent groups (Kruskal Wallis test) and, also, to study differences between 2 independent groups (U Mann-Whitney test). Analysis intrasubject were carried out with Friedmann's test.

For cases in which a normal distribution is presented and the homoscedasticity criterion is met, parametric tests have been performed. Thus, the T-Student test was performed to measure differences between 2 independent groups.

In addition, intrasubject tests and trend analysis have been carried out through repeated measures tests.

The differences have been determined with a confidence value α of 95% and a $p < 0.05$ was considered statistically significant.

Results and Discussion

1. Experiment 1. Previous experiences induce differential responses in Instrumental Successive Negative Contrast procedure in rats.

Those animals that did not learn the task were eliminated from the analyzes. Thus, 8 animals per group were included. The design was 3 (Group: Experimental, Increment, Control) x 10 (sessions). The dependent variable recorded was the time to cross the maze throughout the experiment (Figure 13).

The Shapiro Wilk and Levene tests showed a distribution of the sample that does not follow the normal distribution, although a homocedasticity of the data. For this reason, Kruskall-Wallis and U Mann-Whitney tests have been performed for cross-group analysis and the Friedmann test for intrasubject analyzes.

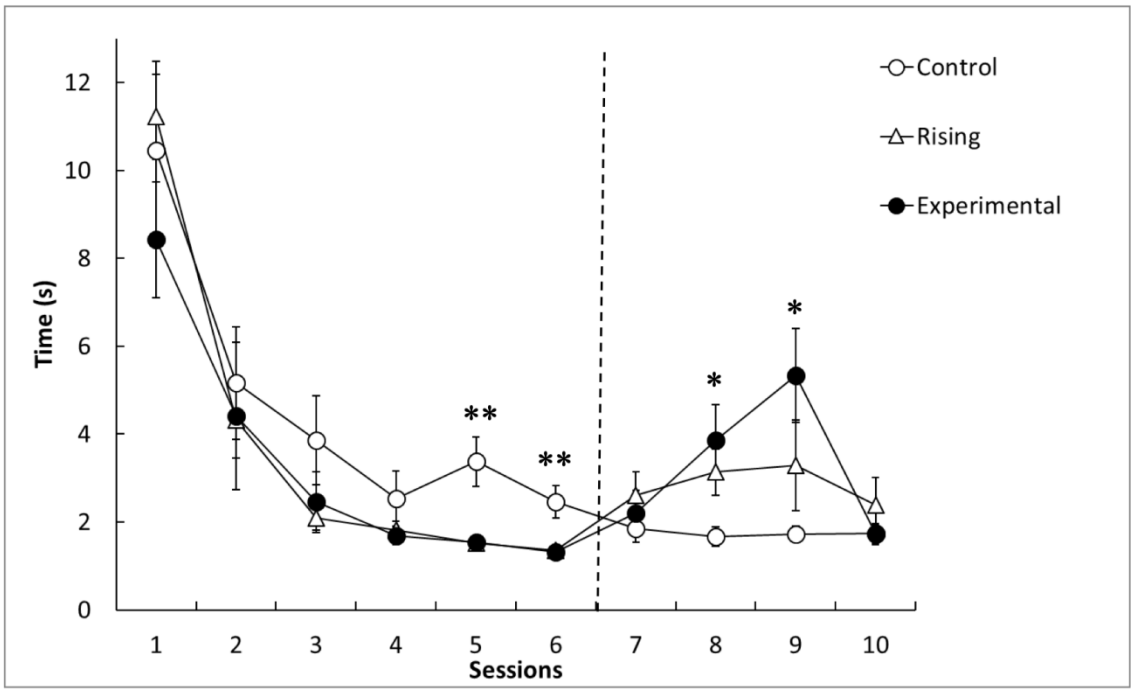

Figure 13. Mean (± SEM) latency (s) to reach the goal-box on each session. The black and white circle represents the experimental group (12–2 pellets) the control group (2–2 pellets), respectively. The white triangle represents the incrementing group (2, 4, 6, 8, 10, 12 – 2 pellets). Each point and vertical line represent the mean ± SEM of values obtained in at least ten animals per group. The dashed line separates the preshift (sessions 1 to 6) and postshift (sessions 7–10) phase. Statistically significant differences between the values and the control group: *p. <0.05 and ** p. <0.01.

Preshift Trials

The preshift phase included the first six days of the study. In this phase, the animals of the experimental group had access to 12 pellets, the control group to 2 pellets and the rising group to 2, 4, 6, 8, 10 and 12 pellets for each day.

Intrasubject analysis showed a day effect in this phase for all groups ($\chi 2$ (5) = 80,929; p. 000). Therefore, there was an effect of days on the running speed to cross the maze, that is, the time to go through the maze was shorter every day.

Intergroup analysis for independent samples showed significant differences in this phase. The Kruskal-Wallis test showed significant differences for day preshift 5 ($\chi 2$ (5) = 9.035; p. 011) and preshift 6 ($\chi 2$ (5) = 11.375; p. 003). Specifically, in preshift 5 differences were found between the incrementing group and the control group (U = 7,000; p. 007) and between the experimental group and the control group (U = 8,000; p. 010), but not between the experimental and incrementing groups (U = 34,000; p. 879). Similarly, in preshift 6 differences were found between the incrementing group and the control group (U = 5,000; p. 003) and between the experimental group and the control group (U = 4,000; p. 002), but not between the experimental and the incrementing group (U = 30,000; p. 879).

Postshift trials

Postshift trials occupied the last four days of the study. During this phase, all animals had access to 2 pellets regardless of the assigned group.

Intrasubject analysis showed a day effect for the experimental group ($\chi 2_{(3)}$ = 8,250; p. 041), but not for the control group ($\chi 2_{(3)}$ = 0.000; p. 1,000) or for the experimental group ($\chi 2_{(3)}$ = 1,050; p. 789). Therefore, there was an increment of the speed throughout the sessions in the experimental group, but not in the control or in the incrementing group.

After that, an independent samples comparison was performed. The Kruskal-Wallis test did not indicate significant differences for postshift day 1 ($\chi 2_{(2)}$ = 1,140; p. 565). On the other hand, differences were found in the postshift day 2 ($\chi 2_{(2)}$ = 7,305; p. 026) and also in the postshift day 3 ($\chi 2$ (2) = 8,235; p. 016). Specifically, in postshift day 2 differences were found between the incrementing group and the control group (U = 54,000; p. 021) and between the experimental group and the control group (U = 54,000; p. 021) but not between the incrementing group and the experimental group (U = 35,000; p. 798). However, in postshift day 3 only a difference was found between the experimental group and the control group (U = 58,000; p. 005).

Polynomial analysis has shown a lineal trend for the control group ($F_{(1,7)}$ = 16.377; p. 005), whereas the trend for the experimental group has been cubic ($F_{(1,7)}$ = 68.518; p .000). The incrementing group has shown a cubic trend but mostly significant for the quadratic trend ($F_{(1,7)}$ = 128.938; p. 000). The polynomial analysis has shown a gradual increase in the travel speed by the control group over the days. However, the experimental group has shown a change in the tendency derived from the manipulation of the reinforcer during the postshift phase. Finally, the incrementing group presents a more complex function derived from previous experience and the devaluation of the reinforcer that have marked several changes in the trend.

Several implications have been determined from the results found in this experiment that will be discussed below.

First of all, the results showed similar performance in experimental group and rising group from the second day of the preshift phase despite the disparity be-tween reinforcers (4 pellets vs 12 pellets). Similarly, both groups (experimental and rising) ran the maze faster than the control group. This disparity was not found until the preshift 6 day by Flaherty et al. (1983). Differences in preshift phase suggest a differential motivation or "drive" to achieve the reinforcer in the groups. Similarly, de-spite the low reinforcer, the expectation of a reinforcer of higher magnitude could enhance the motivation.

Regarding the results found in the postshift phase, (that is, when all the animals have access to a low reinforcer) no type of frustration had been found during the first postshift day (Amsel, 1992; Papini, 2009). However, a similar frustration during the second postshift day had been found in experimental and incrementing groups. Nevertheless, the frustration disappeared in the incrementing group on the third postshift day. The lack of significant differences during the first postshift day might be due to the dimensions of the maze. Animals do not expect the devaluation of the reinforcer during the first postshift day and, for this reason as well as the short distance to run the maze, could apparently reduce the frustration.

Flaherty et al. (1983) argued that the fact that rodents had not developed a great frustration expectation could be due to the experimental paradigm used. In the cSNC the rat is passively exposed to the reinforcer without having done any task. By contrast, the iSNC requires that the rodent performs a task to acquire the reinforcer. However, results

found in iSNC have been quite similar to those observed by Flaherty et al. (1983) excluding the possibility of any procedural inference. Our results support that the animal may be averaging the total amount of enhancer administered during the preshift phase (360 vs 252 pellets, for the experimental and incrementing group, respectively). Likewise, during the devaluation, the experimental group with a greater reinforcer (360 pellets) presented a greater frustration against the group with a gradual rising of the reinforcer (252 pellets) (McHose & Peters, 1975). For this reason, the hypothesis suggested by McHose and Peters (1975) that the animal creates an expectation during the preshift phase has been rejected.

Another variable that might affect the results is the ceiling effect. In the iSNC, there is a ceiling effect in the speed calculated in running the maze (less than 1 second) that it is difficult to improve due to biological causes. Specific circumstances are re-quired to eliminate the ceiling effect (long maze, measurement of other behavioral responses in frustration, etc.).

2. Experiment 2. Frustration induces hypoalgesia.

2.1. Mechanical hypoalgesia induced by reward devaluation

A contrast (32% vs. 4% sucrose) x von Frey (session 17 vs. 18) x Session (1-16) analysis of preshift consummatory performance indicated that groups exposed to 32% consumed significantly more sucrose than groups receiving 4% of sucrose, $F_{(1, 37)}$ = 28.65, $p<0.001$, and also there was a significant increase in consumption across sessions, $F_{(15, 555)} = 87.15$, $p<0.001$. Sucrose consumption during the last preshift session (16) and the three postshift sessions (17-19) is presented in Figure 14. Consumption was higher in groups exposed to 32% sucrose than to 4% sucrose on session 16, the last preshift session, $F_{(1, 37)} = 20.80$, $p<0.001$, but there was no difference between groups assigned for von Frey testing on session 17 or 18, or interaction between these two factors, $Fs < 1.91$, $ps>0.17$. An analysis of postshift sessions data indicated that there was a weak cSNC effect lasting a single session. This analysis yielded a significant interaction between contrast and postshift session, $F_{(2, 74)} = 5.68$, $p<0.006$, and LSD pairwise comparisons confirmed that downshifted groups consumed significantly less sucrose on session 17 than unshifted controls, $F_{(1, 37)} = 4.48$, $p<0.05$. The differences were not significant for postshift sessions 18 and 19. There was no evidence of

deviations from normality on sessions 16-19 (statistics: < 0.18, ps>0.09). Thus, consummatory behavior showed no evidence of a downshift effect on session 18, before the von Frey test was administered in Groups 32/18 and 4/18. Additionally, there was no evidence that von Frey testing in one day affected consummatory behavior in the contrast box the following day.

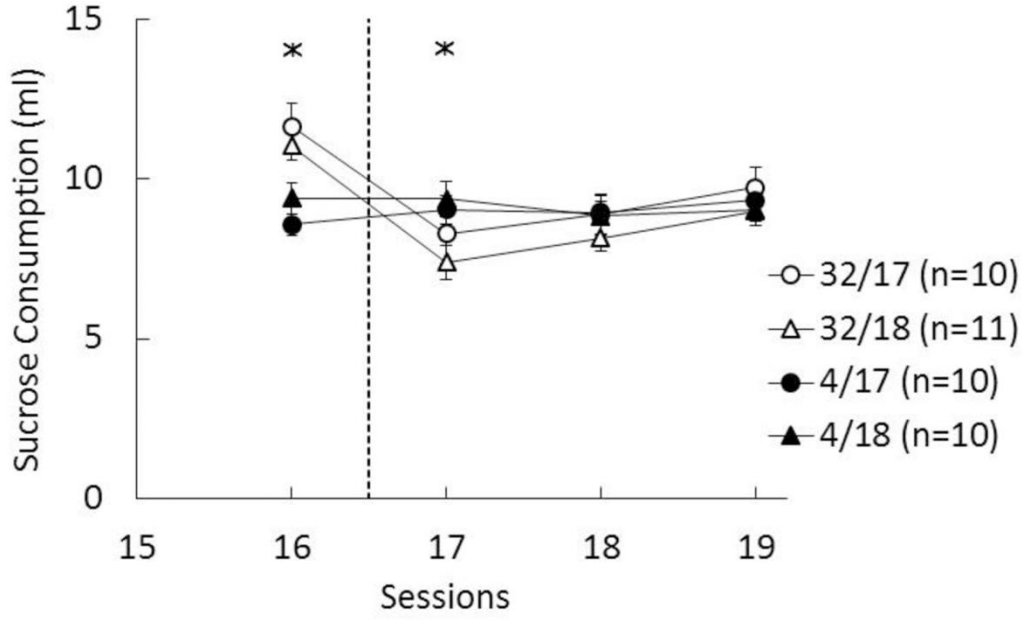

Figure 14. Von Frey test: mean (±SEM) sucrose consumption (ml) during the last preshift session (16) and either session 17 or 18 (Post) depending on the groups. 32: animals exposed to reward devaluation from 32% to 4% sucrose during postshift sessions. 4: animals exposed to an unshifted reward condition, always receiving access to 4% sucrose throughout the experiment. The asterisk reflects a significant difference between both downshifted groups vs. both unshifted controls (see text for details).

Figure 15 shows the mean pain thresholds in groups tested on baseline session, and after session 17 (top) or 18 (bottom). Whereas the baseline measurement was obtained at the same time for all animals before cSNC protocol, the postshift measurements were obtained 5 and 300 min after the contrast session. Thus, these three values (baseline, 5 min, and 300 min) are separated by different time interval. Because the relevant comparisons are between downshifted and unshifted groups tested equally on any given day, except for their prior history, these results were analyzed separately with one-way designs.

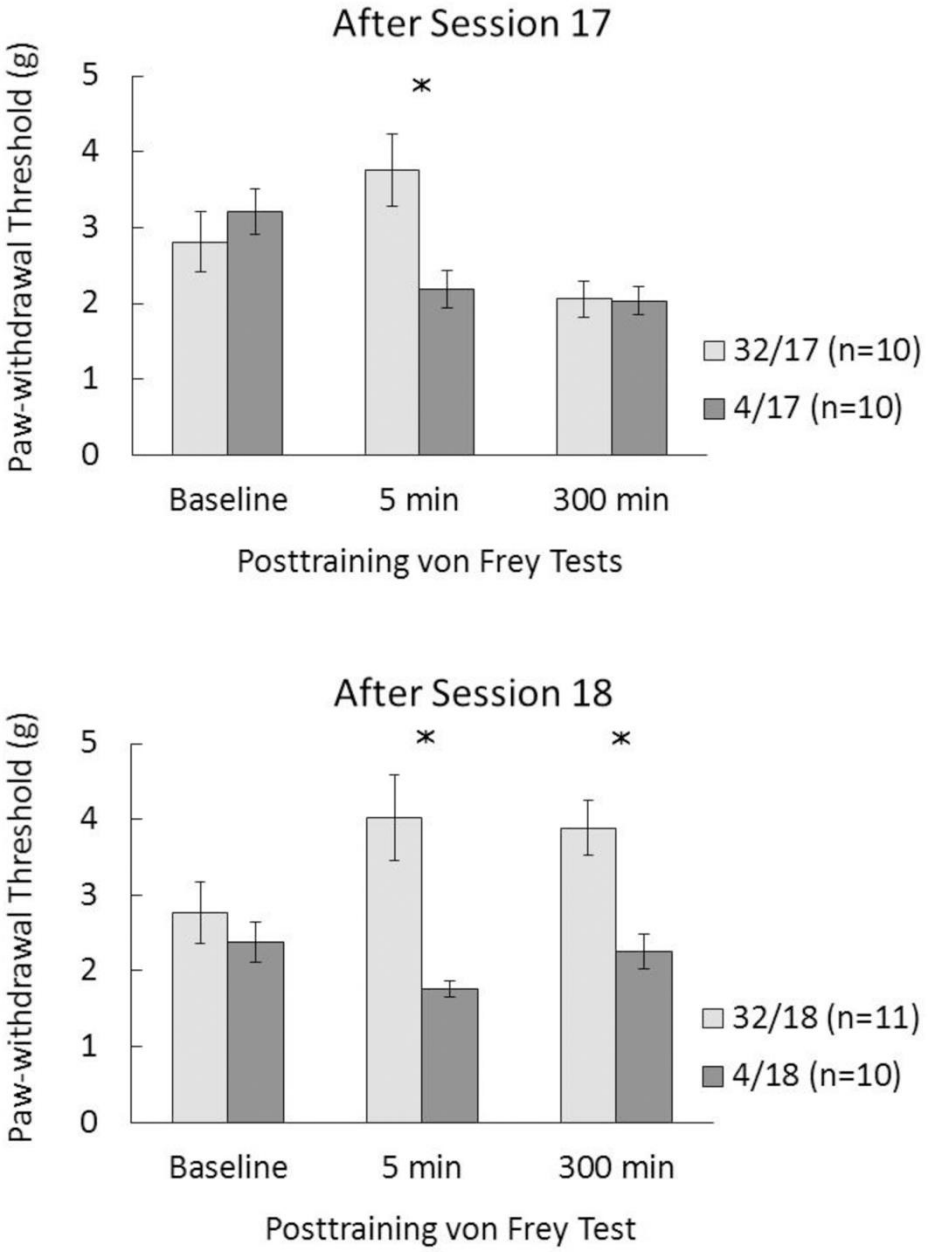

Figure 15. Threshold force (g) for paw withdrawal in the von Frey test during baseline sessions and either 5 or 300 min after sessions 17 (top) or 18 (bottom), depending on the groups. 32: 32-to-4% sucrose downshift. 4: unshifted controls always exposed to 4% sucrose. The asterisks reflect a significant difference between the corresponding downshifted vs. unshifted groups (see text for details).

Baseline measurements did not differ between downshifted and unshifted groups for both test day conditions, Fs < 1. Two main outcomes are observed in Figure 15. First, in groups tested after the first downshift session (32/17, 4/17), pain thresholds increase after 5 min, but they decreased after 300 min to match the values of unshifted controls. This was confirmed statistically. Relative to unshifted controls, downshifted animals exhibited hypoalgesia 5 min after session 17, $F_{(1, 18)} = 8.61$, $p<0.01$, but not 300 min after that session, $F < 1$. Second, in groups tested after the second downshift trial, pain

thresholds also increased in downshifted animals relative to unshifted controls after 5 min, $F_{(1, 19)}$= 14.28, p<0.002, but, unexpectedly, this group difference remained significant even 5 hours after the end of the second downshift session, $F_{(1, 19)}$ = 13.58, p<0.003. No evidence of deviations from normality was detected on any of the significant effects shown in Figure 14 (statistics: < 0.23, ps>0.18).

The results reported above were not dependent on group differences in feeding motivation, as assessed in terms of body weight. The mean (±SEM) weights across sessions 1-19 were 239.6 (5.5), 240.0 (5.6), 232.4 (3.2), and 231.6 (4.1) g for Groups 32/17, 32/18, 4/17, and 4/18, respectively. A Contrast x von Frey analysis detected no effects, all Fs < 1. A similar analysis on the mean weights during postshift sessions 17-19 also detected no effects, all Fs < 1.

2.2. Heat hypoalgesia induced by reward devaluation

A Contrast (32% vs. 4% sucrose) x plantar test (session 17 vs. 18) x Session (1-16) analysis of preshift consummatory performance indicated a significant effect of the variables session F(15,540) = 60,208, p < 0,0001, contrast F(1,36) = 78,145, p < 0,0001 and the interaction contrast x session F(15,540) = 5,215, p < 0,0001.

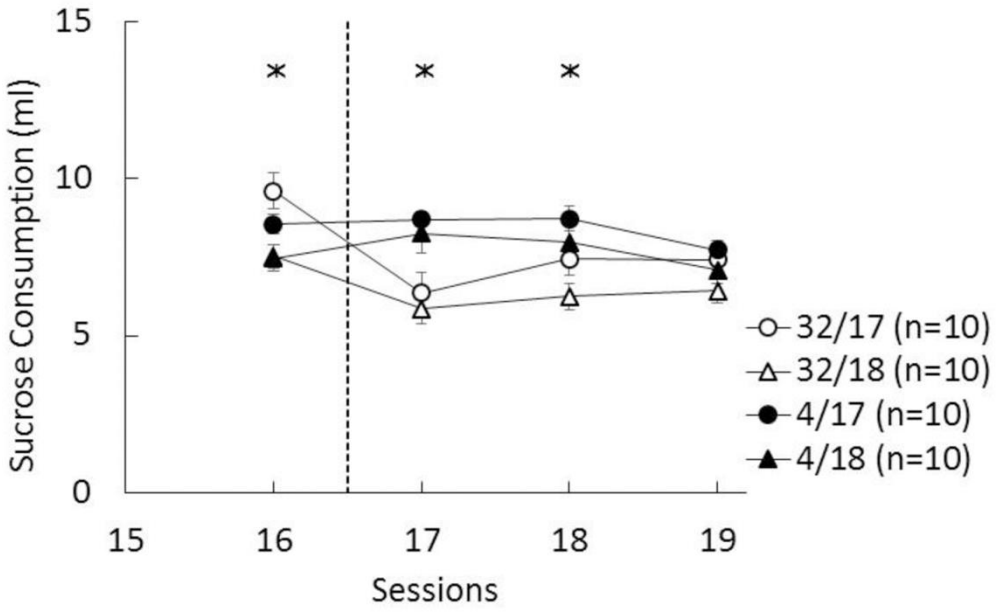

Figure 16. Hargreaves test: mean (±SEM) sucrose consumption (ml) during the last preshift session (16) and either session 17 or 18 (Post) depending on the groups. 32: animals exposed to reward devaluation from 32% to 4% sucrose during postshift sessions. 4: animals exposed to an unshifted reward condition, always receiving access to 4% sucrose throughout the experiment. The asterisk reflects a significant difference between both downshifted groups vs. both unshifted controls (see text for details).

Interaction analysis showed significant differences between control and contrast groups in the sucrose consumption in each session (Fs > 10.8; $p<0.002$). Moreover, control ($F = 23.26$, $p<0.0001$) and contrast ($F = 60.99$, $p<0.0001$) groups showed an increase in the consumption throughout the sessions. Sucrose consumption during the last preshift session (16) and the three postshift sessions (17-19) is presented in Figure 16. Consumption was higher in groups exposed to 32% sucrose than to 4% sucrose on session 16, the last preshift session, $F(1, 36) = 11.853$, $p<0.01$, but there was no difference between groups assigned for plantar testing on session 17 or 18, or interaction between these two factors, Fs < 2.22, ps>0.14.

An analysis of postshift sessions data indicated that there was a cSNC effect. This analysis yielded a significant interaction between contrast and postshift session, $F_{(2, 73)} = 30$, $p<0.002$. Interaction analysis showed a cSNC (downshifted groups consumed significantly less sucrose than unshifted controls) before the plantar test was administered on session 17 $F_{(1, 36)} = 22.93$, $p<0.0001$ and session 18 $F_{(1, 36)} = 10.62$, $p<0.002$. Consummatory behavior showed no evidence of a downshift effect on session 19. There was no evidence of deviations from normality on sessions 16-19 (statistics: < 0.909, ps>0.072).

Figure 17 shows the heat-pain threshold in groups tested on baseline session, and after session 17 (top) or 18 (bottom). Whereas the baseline measurement was obtained at the same time for all animals before cSNC protocol, the postshift measurements were obtained 15 and 300 min after the contrast session. Thus, these three values (baseline, 15 min, and 300 min) are separated by different time interval. Because the relevant comparisons are between downshifted and unshifted groups tested equally on any given day, except for their prior history, these results were analyzed separately with one-way designs.

Baseline measurements did not differ between downshifted and unshifted groups for both test day conditions, Fs < 0.84. Two main outcomes are observed in Figure 17. First, in groups tested after the first downshift session (32/17, 4/17), pain thresholds increase after 15 min, but they decreased after 300 min to match the values of unshifted controls. This was confirmed statistically. Relative to unshifted controls, downshifted animals exhibited hypoalgesia 15 min after session 17, $F_{(1, 18)} = 6.42$, $p<0.02$, but not 300 min after that session, $F < 0.53$. Second, in groups tested after the second downshift trial, pain thresholds also increased in downshifted animals relative to unshifted controls

after 15 min, $F(1, 18) = 38.18$, $p<0.0001$, but, unexpectedly again, this group difference remained significant even 300 min after the end of the second downshift session, $F(1, 18) = 20.43$, $p<0.0001$. No evidence of deviations from normality was detected on any of the significant effects shown in Figure 16 (statistics: < 0.0.91, ps>0.07).

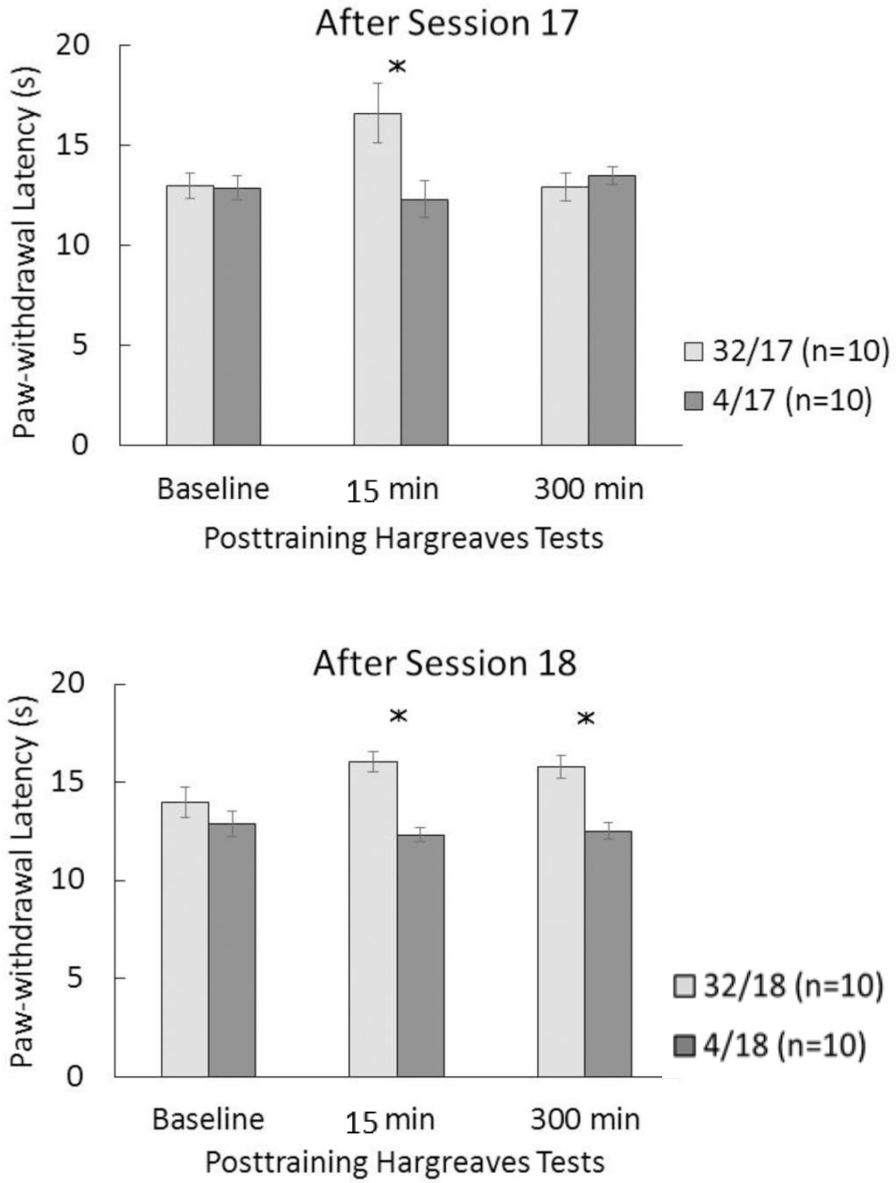

Figure 17. Paw-withdrawal latencies (s) recorded during the Hargreaves test administered in baseline, and 15 or 300 min after sessions 17 (top) or 18 (bottom), depending on the groups. 32: 32-to-4% sucrose downshift. 4: unshifted controls always exposed to 4% sucrose. The asterisks reflect a significant difference between the corresponding downshifted vs. unshifted groups (see text for details).

2.3.Discussion about the hypoalgesia data

Three aspects of the present results merit discussion: (1) the relatively weak contrast effect both experiments; (2) the issue of trial selectivity of the effects of reward

devaluation on pain sensitivity; and (3) the postsession time course of these effects. We discuss below each of these in turn.

First, the cSNC effect observed in both experiments was rather weak, compared to typical results in this task. Typically, Flaherty (1996) the cSNC effect lasts between 1-5 sessions after the downshift, whereas in this case it lasted a single session and the difference between downshifted and unshifted groups was relatively small in absolute terms.

This protocol was carried out after an iSNC with ethanol intake. Before the manipulation, animals were counterbalanced in the groups to eliminate the effect of the type of previously manipulation. Prior experience in a related task, iSNC, might have reduced the emotional impact of the 32-to-4% sucrose downshift in the present experiment. In Roman low-avoidance rats, which typically behave similarly to non-selected Wistars in SNC tasks (Papini et al., 2015; Torres & Sabariego, 2014), prior downshift experience in the iSNC task eliminated the cSNC effect in a subsequent phase (Cuenya et al., 2015). To test for this possibility, postshift consummatory behavior was analyzed again with the addition of a factor identifying whether the animal had prior downshift or unshift experience in the iSNC situation. The analysis yielded the same contrast by session significant interaction reported above, but none of the factors (main or interaction effects) involving prior experience was significant, (Von Frey experiment: $Fs < 1.94$, $ps > 0.17$; Plantar test experiment: $Fs < 2.475$, $ps > 0.13$). In addition, we evaluated the potential effect of prior exposure to 2% ethanol during the iSNC experiment on the cSNC effect with Contrast x Ethanol x Session analyses for the preshift and postshift data. There was no evidence of a main effect of ethanol or of any interaction with contrast in these results, (von Frey experiment: $Fs < 3.08$, $ps > 0.05$; Plantar test experiment: $Fs < 3.308$, $ps > 0.08$). Thus, there was no evidence that prior downshift or ethanol experience had a measurable effect on the size of the cSNC effect obtained in this experiment. There are individual differences in the extent to which rats respond to the downshift event in the cSNC situation (Ortega et al., 2014; Papini et al., 2014) that are responsible for variability across experiments. What seems clear is that a weak cSNC effect did not prevent hypoalgesia. Moreover, hypoalgesia was present after session 18 in the von Frey assessment, which had produced no evidence of contrast. Recent research in a different paradigm shows a similar decoupling of measures. Rats exhibit enhanced preference for ethanol and chlordiazepoxide over water immediately

after a reward devaluation experience. Interestingly, preference for these anti-anxiety substances persists longer than the cSNC effect (Manzo et al., 2015). Results such as these suggest that even when animals appear to be behaviorally recovered from the negative effects of reward devaluation on consummatory behavior, other measures (including pain sensitivity in the present experiment) indicate that they are still emotionally aroused.

Second, the issue of trial selectivity was raised by the results of an experiment similar to the present one, except for using the hot plate as an assay assessing pain sensitivity (Mustaca and Papini, 2005). In that experiment, hypoalgesia emerged after the second downshift session, but it was not detected after the first downshift session. A novel aspect of the present results is the finding that hypoalgesia was observed immediately after the first and second downshift sessions, thus providing no support for trial selectivity. These results suggest that trial selectivity may depend on the specific technique used to determine the animal's sensitivity to physical pain. However, the hypoalgesia recorded in the present research was founded using mechanical and thermal pain (in this second case, as was used for the hot plate test). It means that the differential methods for pain threshold detection (mechanical and thermal) applied in this research support that the hypoalgesia observed after the frustration experience is independent to the peripheral pain receptors activated in the both tests applied. Although both tests activate potassium channels, thermal stimuli (plantar test) stimulate the transient receptor-potential channels, whereas mechanical stimuli (von Frey test) stimulate acid-sensitive ion channels and ion channels of the degenerin family (Basbaum et al., 2009) However, these studies differ in some parameters. For example, whereas rats had a single exposure to the hot plate in the experiment just described, they had baseline testing with von Frey filaments and plantar test before key testing after reward devaluation in the present experiment.

On the other hand, von Frey test and plantar test estimates pain thresholds in terms of paw withdrawal applying a mechanical or thermal localized stimulus, whereas hot plate test imply the stimulation of several receptors (Le Bars et al., 2001). It produces that the absence of hypoalgesia observed after the first downshift session in the hot plate test respect von Frey and plantar test might be produced for a less sensitivity of this test. It is especially relevant if considered that hypoalgesia observed after the first downshift session looks be less intense either in von Frey test or plantar test. Furthermore, whereas

the current data suggest that the detection threshold is similar after the first and second downshift sessions, it is possible that mechanical (von Frey) and thermal (hot plate) procedures have a different threshold for expression of hypoalgesia. There seem to be no explicit comparison assessing the relative power of these two techniques. Moreover, the hot plate procedure typically involves exposure to a single temperature, whereas the von Frey test is designed to detect the lower threshold of pain sensitivity. This fact suggesting that von Frey and plantar test are more sensitive techniques. These differences might explain the detection of hypoalgesia after the first downshift session in the present experiment. A comparison between different thermal (e.g., hot plate, tail flick, Hargreaves test), mechanical (e.g., Randall-Selitto, von Frey test) and chemical (e.g., formalin, writhing test) assays may provide additional insights about the relative sensitivity of various methods for detecting hypoalgesia after reward devaluation (Basbaum et al., 2009). A similar situation was described for the relationship between reward devaluation and plasma corticosterone levels. Initially it was reported that corticosterone was elevated after the second downshift session, but not after the first downshift session (Mulder & Pritchett, 2004). However additional measurements with a different procedure detected differences after both sessions (Mogil et al., 2001).

Third, studying the postshift course of hypoalgesia produced an unexpected outcome: Elevated pain thresholds 5 hours after the end of the second downshift session. There are at least two possible explanations for this 300-min hypoalgesia effect, both relaying on a repeated exposure to the downshift event (i.e., after two, rather than one, downshift sessions). It is possible that a second exposure to the downshifted reward caused a degree of emotional arousal that did not completely decay in the following 5 h. In fact, given that the level of hypoalgesia after the second downshift session after 5 (or 15) vs. 300 min is virtually identical, one would have to assume that there was no decay whatsoever of emotional activation. This is difficult to substantiate since by a variety of measures, the consequences of reward omission, whether behavioral or physiological, seem to decay rather sharply in time (i.e., in the order of seconds to minutes, depending on the situation; Mitchell & Flaherty, 1998; Pecorano et al., 2009). As mentioned above, postshift treatments that improve the aversive memory of the downshift failed to affect cSNC if administered after a long delay. For example, corticosterone administered immediately after the first downshift session prolonged the cSNC effect in subsequent sessions, but the effect dissipated when corticosterone was administered 3 h

after the first postshift session (Bentosela et al., 2006). Moreover, plasma corticosterone elevation was reported 10 and 20 min, but not 40 min after the second reward downshift session (Mulder & Pritchett, 2004). Although the effects of reward devaluation do not seem to persist for several hours, the response to reward devaluation can be modulated by events that occurred hours earlier. In one experiment (Dudley & Papini, 1995), a 5-min access to a novel open field 6 h before reward devaluation reduced the level of consummatory suppression (i.e., downshifted rats consumed more sucrose than a group lacking open-field exposure). Interestingly, this effect was observed with 1-h and 6-h intervals, but not with a 3-h interval or when open-field exposure occurred immediately before reward devaluation, suggesting a complex temporal dynamic.

The 300-min hypoalgesia effect could also depend on contextual reactivation of the devaluation event because of common features between the contrast and von Frey boxes. Consummatory behavior in the cSNC task can come under contextual control (Stout et al., 2003), thus providing a possible mechanism. Some of the common elements involve the room in which both contrast and pain threshold tests are carried out, and the clear Plexiglas walls of both boxes. This hypothesis assumes that a minimum number of two sessions of exposure to the reward devaluation event is necessary for the 300-min hypoalgesia effect to occur. If the effect depends on some minimum amount of exposure, then a more extensive duration for the first devaluation session should also lead to this 300-min hypoalgesia effect. For example, the effects of the GABAergic anxiolytic chlordiazepoxide on cSNC are not observed during the first devaluation session when it lasts 5 min, but they emerge later in that session if its duration is extended to 10 min (Justel et al., 2014). Moreover, because the cSNC effect is transient, complete recovery from reward devaluation should eliminate the 300-min hypoalgesia effect. These hypotheses remain to be evaluated empirically.

The present results demonstrate the influence that psychological pain induced by reward devaluation can have over sensitivity to physical pain. Although the neurobiological mechanisms underlying such modulation are partially known (Papini et al., 2015), a more complete understanding awaits further study.

3. Experiment 3. Role of sigma-1 receptors ligands on the frustration induced by the consummatory Successive Negative Contrast procedure

The last experiment of the present Doctoral Thesis has studied the effect of the administration of different Sig1R agonists and antagonists using the cSNC procedure. In addition, a control group went through the entire procedure with the administration of the vehicle drug (saline) In all cases the administration occurred in a single trial 30 minutes before the first postshift session.

Firstly, the results obtained in the groups administered with the vehicle are presented. Subsequently, the results obtained with the Sig1R antagonists and, finally, the Sig1R agonists effects are shown.

The study of the form and variability has indicated that both groups follow a normal distribution and meet the criteria of homocedasticity of the sample (p.> 0.05).

The sucrose consumption mean has been represented in the Figure 18. The analysis between groups for two independent samples have found differences between the groups in the preshift session 9 (t = -3.053, p. 0.009), in the first postshift session (t = 2.207, p. 0.45) and in the third postshift session (t = 2, 424, p. 0. 30).

As the intrasubject results obtained in Experiment 1, polynomial analysis indicated a lineal trend for the control group ($F_{(1,7)}$ = 30,604; p. 001), however, a quadratic trend for the experimental group was obtained ($F_{(1.7)}$ = 11.346; p.012).

Results showed a contrast effect of the shifted group compare to the unshifted group which lasted for 3 days. Therefore, the manipulation of the reinforcer produces an effect in the behavioral response which means a frustration perceived in the shifted group and this frustration was independent of the vehicle administration. That is, there is a frustration effect was independent of the vehicle administration

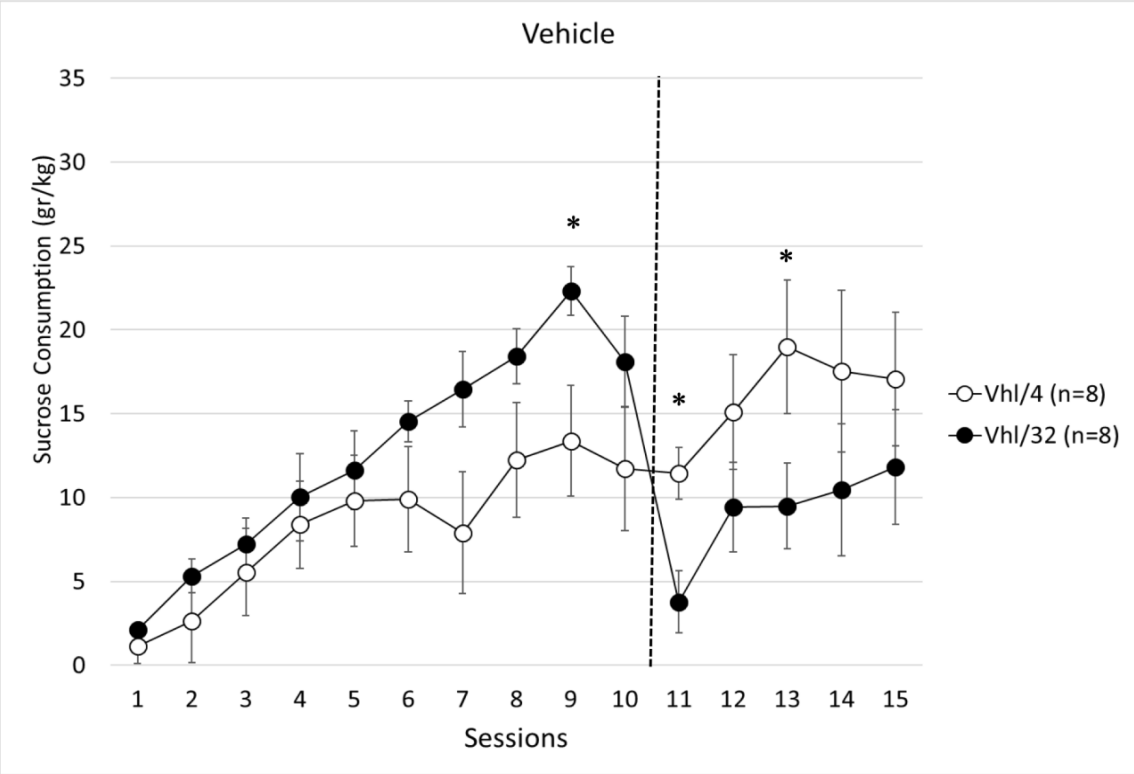

Figure 18. Mean (±SEM) sucrose consumption (gr/kg) during the cSNC procedure for vehicle administrations groups depending on the shifted groups. 32: animals exposed to reward devaluation from 32% to 4% sucrose during postshift sessions. 4: animals exposed to an unshifted reward condition, always receiving access to 4% sucrose throughout the experiment. The dashed line separates the preshift (sessions 1 to 10) and postshift (sessions 11–15) phase. The asterisk reflects a significant difference between both downshifted groups vs. both unshifted controls (see text for details).

3.1. Effects of sigma-1 receptor antagonists

The normality and homoscedasticity tests indicated parameters of normality and equality of variances for all groups treated with the Sig1R antagonists, S1RA and BD1063.

Administration of S1RA in consummatory Successive Negative Contrast

The effects of the s.c. administration of S1RA are shown in Figure 19. The results of 64 mg/kg, 32 mg/kg and 16 mg/kg are represented in Figure 19.A, 19.B and 19.C, respectively. The administration of S1RA was always performed 30 minutes before the test on postshift day 1. In all figures are shown the last day of preshift phase (10) and the first three days of postshift phase (11, 12 and 13) (for more information, see Annex 1).

The results between groups for the dose 64 mg/kg have shown significant differences on the preshift days 9 (t = -5,252, p.0.00) and 10 (t = -3,688, p.0.002) and postshift 1 (t = 4,438, p. 0.00). The intrasubject analysis showed a lineal trend for the control group ($F_{(1,7)}$ = 35,274; p.001) and a quadratic ($F_{(1,7)}$ = 14,304; p. 005) and cubic trend, but mostly significant for the cubic trend ($F_{(1,7)}$ = 17,858; p. 003) for the experimental group. The interactions between groups treated with 32 mg/kg of S1RA indicated significant differences only on postshift 1 (t = 5,098, p. 000). Similarly, to the higher dose, the intrasubject analysis showed a lineal trend for the control group ($F_{(1,7)}$ = 41.805; p.001) and a quadratic ($F_{(1,7)}$ = 10.431; p. 014) and cubic trend, but mostly significant for the cubic trend ($F_{(1,7)}$ = 17,091; p. 004) for the experimental group. The effects of 16 mg/kg showed significant differences between the groups in postshift 1 (t = 3.155, p.0.008) and postshift 2 (t = 2.745, p. 0.018). The intrasubject analysis showed a lineal trend for the control group (F (1.7) = 51,491; p,000) and a quadratic trend (F (1,7) = 10,000; p. 020) for the experimental group.

The results obtained with S1RA suggest that the higher doses (or 64 mg/kg and 32 mg/kg) reduces the contrast only on the second postshift day, but not on the first postshift day. The lower dose 16 mg/kg) seems to have no effect on contrast at any period. However, the similar sucrose consumption in preshift 10 could have influenced the effects induced by 16 mg/kg. Overall, the data obtained with S1RA suggest that this compound does not affect the contrast on the first postshift day, whereas only the high doses seem to reduce the contrast on the second postshift day.

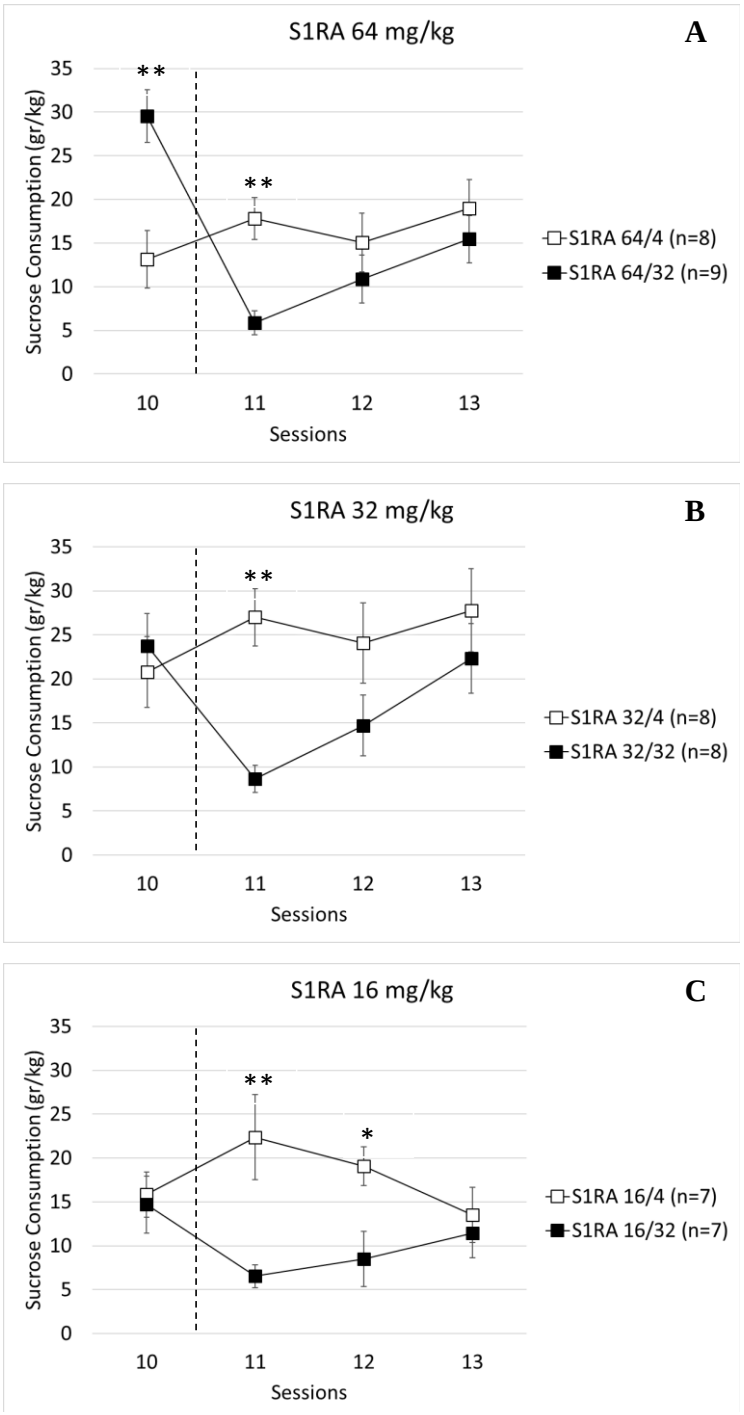

Figure 19. S1RA administration in Consummatory Successive Negative Contrast. Figure shows MEAN (± SEM) of sucrose consumption in the cSNC protocol after S1RA administration. Drug dose (64, 32 or 16 mg/kg)/32: animals exposed to reward devaluation from 32% to 4% sucrose during postshift sessions. Drug dose (64, 32 or 16 mg/kg)/4: animals exposed to an unshifted reward condition, always receiving access to 4% sucrose throughout the experiment. (A) The effect of S1RA 64 mg/kg showed a contrast during postshift 1 in shifted group compare to unshifted group. Frustration lasted one day due to the absence of differences between groups in postshift 2. (B) The effect of 32 mg/kg showed a similar result than in 64 mg/kg (C) The effect of S1RA 16 mg/kg showed a contrast during postshift 1 and 2 in shifted group compare to unshifted group. Frustration lasted two days due to the absence of differences between groups in postshift 3. The dashed line separates the preshift (day 10) and postshift (days 11–13) sessions. Statistically significant differences between the animals exposed to a high (32 %) and low (4 %) reinforcer: *p. <0.05 and ** p. <0.01.

Administration of BD1063 in Consummatory Successive Negative Contrast

The results of the s.c. administration of BD1063 are shown in Figure 20. Figure 20.A, 20.B and 20.C represent the results of 16 mg/kg, 4 mg/kg and 1 mg/kg, respectively. BD1063 was administered 30 minutes before the test on postshift 1. In all figures are shown the last day of preshift phase (10) and the first three days of postshift phase (11, 12 and 13) (for more information, see Annex 2).

The results between groups for the dose 16 mg /kg have shown a borderline significant difference in the preshift days 9 (t = -2, 171, p. 054) and statistical differences between groups in the preshift 10 (t = -4,715, p. 0 00). No differences were found in the postshift phase. The intrasubject analysis showed a lineal trend for the control group ($F_{(1,7)}$ = 12.249; p.010) and for the experimental group ($F_{(1,7)}$ = 17,295; p. 004). These results suggest that BD1063 16 mg/kg completely eliminates contrast.

The interactions between groups treated with 4 mg/kg of BD1063 indicated significant differences only on postshift 1 (U = 34,000; p. 001). The intrasubject analysis showed a lineal trend for the control group ($F_{(1,7)}$ = 23,863; p.003) and significantly quadratic tend ($F_{(1,7)}$ = 80,178; p. 000) for experimental group. These results suggest that BD1063 4mg/kg has no effect in the successive negative contrast.

The effects of 1 mg/kg showed significant differences between the groups in postshift 10 (t=-2,137, p. 052). The intrasubject analysis showed a lineal trend for the control group ($F_{(1,7)}$ = 37,401; p.001) as well as for the experimental group ($F_{(1,7)}$ = 61,098; p. 000). A non-homogeneity of variances has been found in postshift 1 (F=5,702, p. 0.033). Thus, the administration of BD1063 1 mg/kg reverse the successive negative contrast effect.

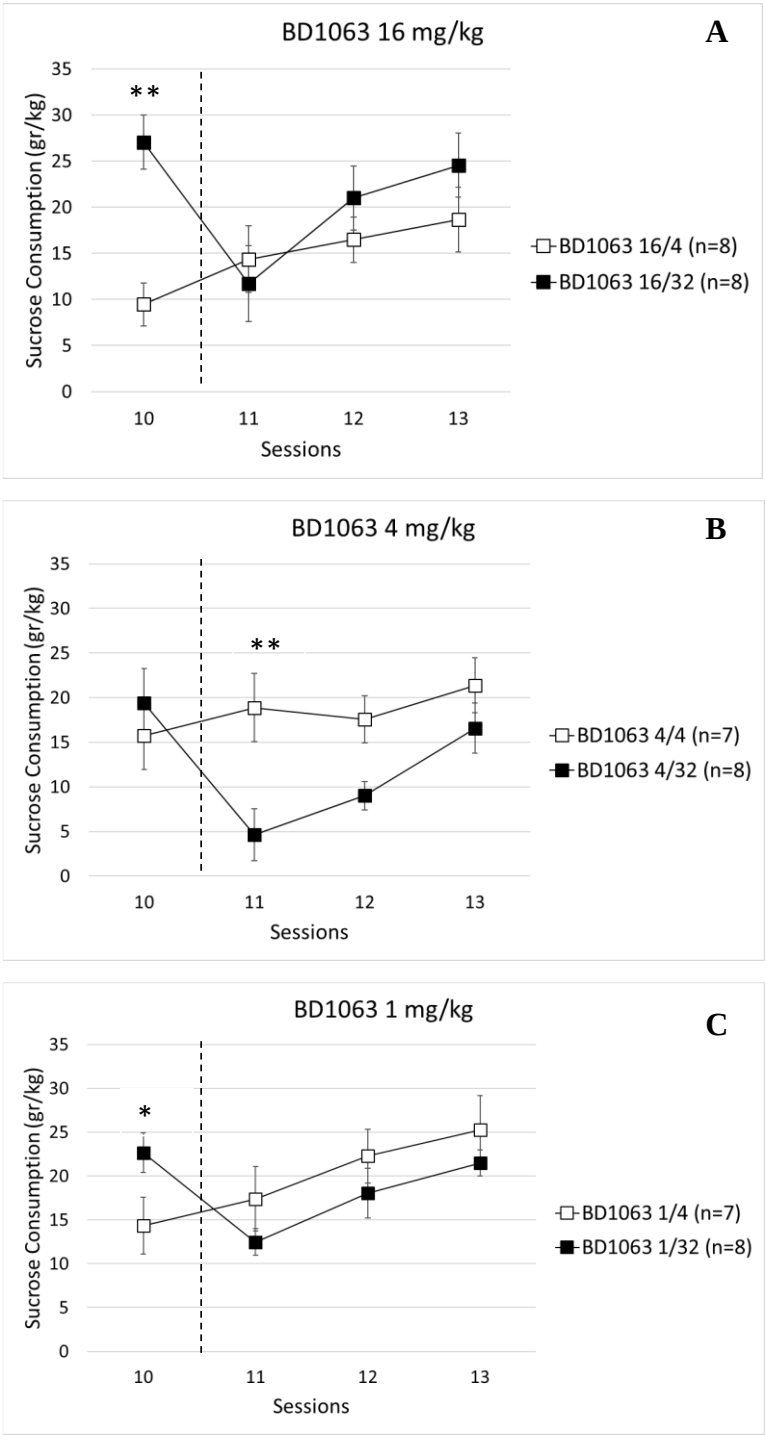

Figure 20. BD1063 administration in Consummatory Successive Negative Contrast (cSNC). Figure shows MEAN ± SEM of sucrose consumption in the cSNC protocol after an BD1063 antagonist administration. . Drug dose (16, 4 or 1 mg/kg)/32: animals exposed to reward devaluation from 32% to 4% sucrose during postshift sessions. Drug dose (16, 4 or 1 mg/kg)/4: animals exposed to an unshifted reward condition, always receiving access to 4% sucrose throughout the experiment. (A) The effect of BD1063 16 mg/kg showed no differences between groups during postshift phase. The administration of this dose eliminated the frustration. (B) The effect of BD1063 4 mg/kg showed contrast for 1 day (postshift 1) (C) The effect of BD1063 1 mg/kg showed similar results than 16 mg/kg. The dashed line separates the preshift (day 10) and postshift (days 11–13) sessions. Statistically significant differences between the animals exposed to a high (32 %) and low (4 %) reinforcer: *p. <0.05 and ** p. <0.01.

BD1063 has shown a "U" shape dose-effect pattern. Specifically, the high and the low doses of BD1063 (16 mg/kg and 1 mg/kg) have reduced or eliminated the contrast in postshift 1 and postshift 2.

Sig1R antagonists have a different response according the specific drug administrated. S1RA has shown a weak dose-dependent effect whereas BD1063 have shown a "U" shape dose-response. According to the Flaherty's formula to study the drug effectivity ratio (DER) in the CSNC, the effects of the Sig1Rantagonists are represented in Figure 21.

DER results suggest the effectiveness of BD1063 in doses of 1 mg/kg. However, according to Flaherty (1996), that effectivity is lower to the found in morphine considering that a score of 1.5 is considered as a good efficacy in the paradigm. Differences between groups before the shift day could be involved in these outcomes.

All of these results (DER and behavioral results) suggest that antagonist of Sig1R has no effect in contrast but BD1063 1 mg/kg seems to reduce it.

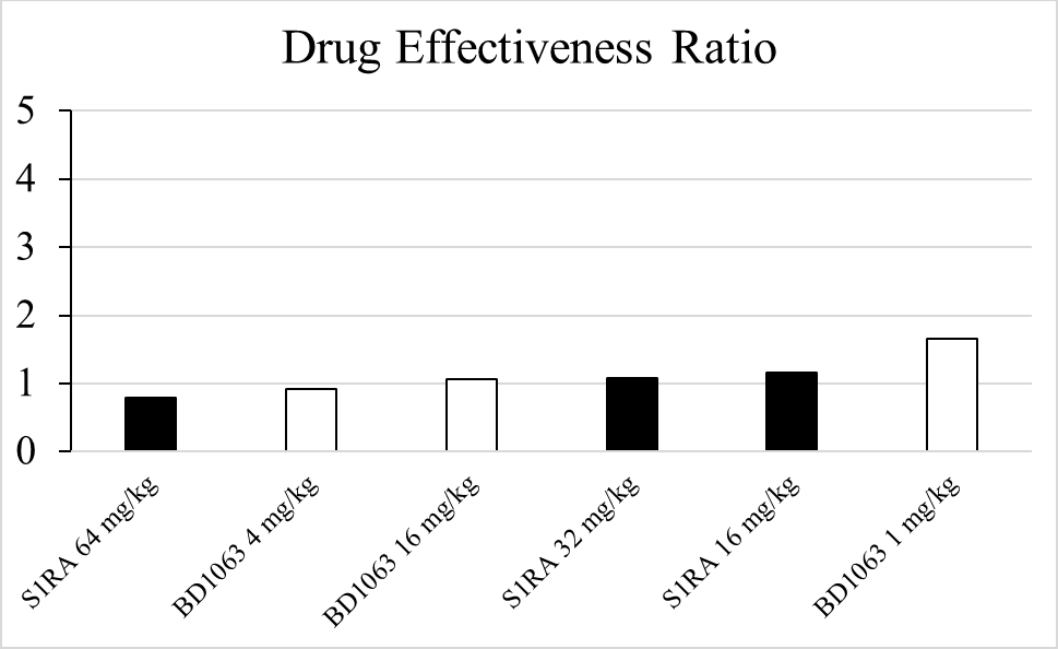

Figure 21. Drug Effectiveness Ratio of Sig1R antagonists S1RA and BD1063. DER shows the efficacy of antagonist drugs in increasing order. BD1063 1 mg has indicated a completely elimination of frustration. While S1RA 16 mg and 32 mg have shown a reduction of frustration in postshift 1.

3.2. Effects of sigma-1 receptors agonists

The normality and homoscedasticity tests indicated parameters of normality and equality of variances for all groups treated with the Sig1R agonists, PRE-084 4 and 8 mg/kg and igmesine and 16 mg/kg. However, non-parametric analysis has been performed to PRE-084 2 mg/kg and igmesine 1 mg/kg due to their parameters shows a non-normality and differences of variances.

Administration of PRE-084 in Consummatory Successive Negative Contrast

The effect of the s.c. administration of PRE-084 is shown in Figure 22. The results of 8 mg/kg, 4 mg/kg and 2 mg/kg are represented in Figure 22.A, 22.B and 22.C, respectively. The administration of PRE-084 was always performed 30 minutes before the test on postshift day 1. In all figures are shown the last day of preshift phase (10) and the first three days of postshift phase (11, 12 and 13) (for more information, see Annex 3).

No statistically significant differences were found between groups at any dose tested (p. >05). Only PRE-084 2 mg/kg exhibited differences in preshift 8 session (U=46,000, p. 0.04). Similarly, polynomial analysis demonstrated that control group and experimental group have a lineal tend ($p.\leq 0.05$) for all the doses (8, 4 and 2 mg/kg).

All doses of PRE-084 seem to eliminate the contrast. The anxiolytic effect of this Sig1R agonist has been demonstrated in other studies (Maurice et al., 2003). Papini (2009) has suggested that the first postshift session day has a memory process that implies the comparison of the differences between preshift and postshift responses. In this line of thought, Maurice and coworkers (1998) point out the improvement in memory of PRE-084 in an experimental Alzheimer model (Maurice et al., 1998). Evidences found in the cSNC paradigm have suggested an interaction of memory during frustration (Ortega et al., 2014; Papini, 2009).

However, since there were no differences before the contrast (same or similar preshift values for vehicle-and PRE-084-treated animals were obtained on day 10), it is difficult to know if the contrast has been attenuated for the drug action or due to possible problems with the learning task.

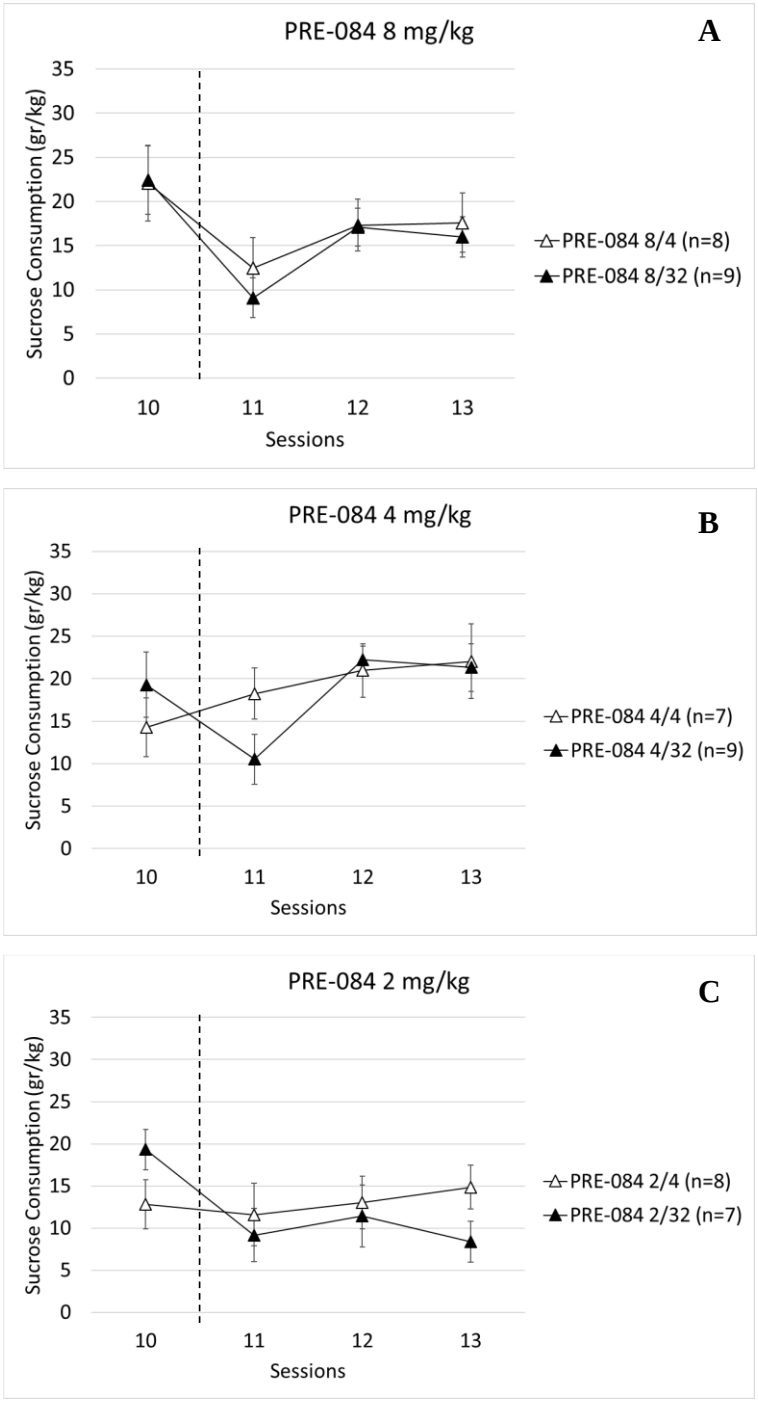

Figure 22.PRE-084 administration in Consummatory Successive Negative Contrast Figure shows MEAN (± SEM) of sucrose consumption in the cSNC protocol after PRE-084 administration. Drug dose (8, 4 y 2 mg/kg)/32: animals exposed to reward devaluation from 32% to 4% sucrose during postshift sessions. Drug dose (8, 4 y 2 mg/kg)/4: animals exposed to an unshifted reward condition, always receiving access to 4% sucrose throughout the experiment. (A) The effect of PRE-084 8 mg/kg showed no differences between groups during postshift phase. The administration of this dose eliminated the frustration. (B) The effect of PRE-084 4 mg/kg showed similar results than 8 mg. (C) The effect of PRE-084 2 mg/kg showed similar results than other. Results suggest the elimination of contrast regardless the dose. The dashed line separates the preshift (day 10) and postshift (days 11–13) sessions. Statistically significant differences between the animals exposed to a high (32 %) and low (4 %) reinforcer: *p. <0.05 and ** p. <0.01

Administration of igmesine in consummatory Successive Negative Contrast

The effect of the s.c. administration of igmesine is shown in Figure 23. The results of 16 mg/kg, 4 mg/kg and 1 mg/kg are represented in Figure 23.A, 23.B and 23.C, respectively. The administration of igmesine was always performed 30 minutes before the test on postshift day 1. In all figures are shown the last day of preshift phase (10) and the first three days of postshift phase (11, 12 and 13) (for more information, see Annex 4).

The administration of 16 mg/kg obtained differences only in preshift 9 (t=-3,877, p. 0.002). By contrast, no differences were found in the postshift phase. The intrasubject analysis showed a lineal trend for the control group ($F_{(1,7)}$ = 15,348; p.008) and a significantly quadratic tend ($F_{(1,7)}$ = 25,537; p. 002) for the experimental group. These results suggest that igmesine 16 mg/kg attenuates, but not eliminates, the contrast.

The effect of igmesine 4 mg/kg exhibited differences in postshift 1 (U=12,000, p. 0.021) and in the second postshift day (t=2,746, p. 0.015). Levene's test showed a p. >0.05 during all sessions, assuming that the variances are equals but not in the first postshift day ($F_{(1,15)}$=6,258, p. 0.024). The intrasubject analysis indicated a lineal trend for the control group ($F_{(1,7)}$ = 39,920; p.000) as well as for the experimental group ($F_{(1,7)}$ = 91,613; p. 002). These results suggest that igmesine 4 mg/kg reduce slightly the contrast.

The interactions between groups after the administration of 1 mg/kg showed differences in preshift 8 (U=45,000, p. 0.007), preshift 9 (U=47,000, p. 0.002), preshift 10 (U=46,000, p. 0.004) and the second postshift day (U=42,000, p. 0.026). A lineal trend for the control group ($F_{(1,7)}$ = 18,197; p.005) and the experimental group ($F_{(1,7)}$ = 78,337; p. 000) was found after an intrasubject analysis. These results suggest that igmesine 1 mg/kg eliminates the contrast.

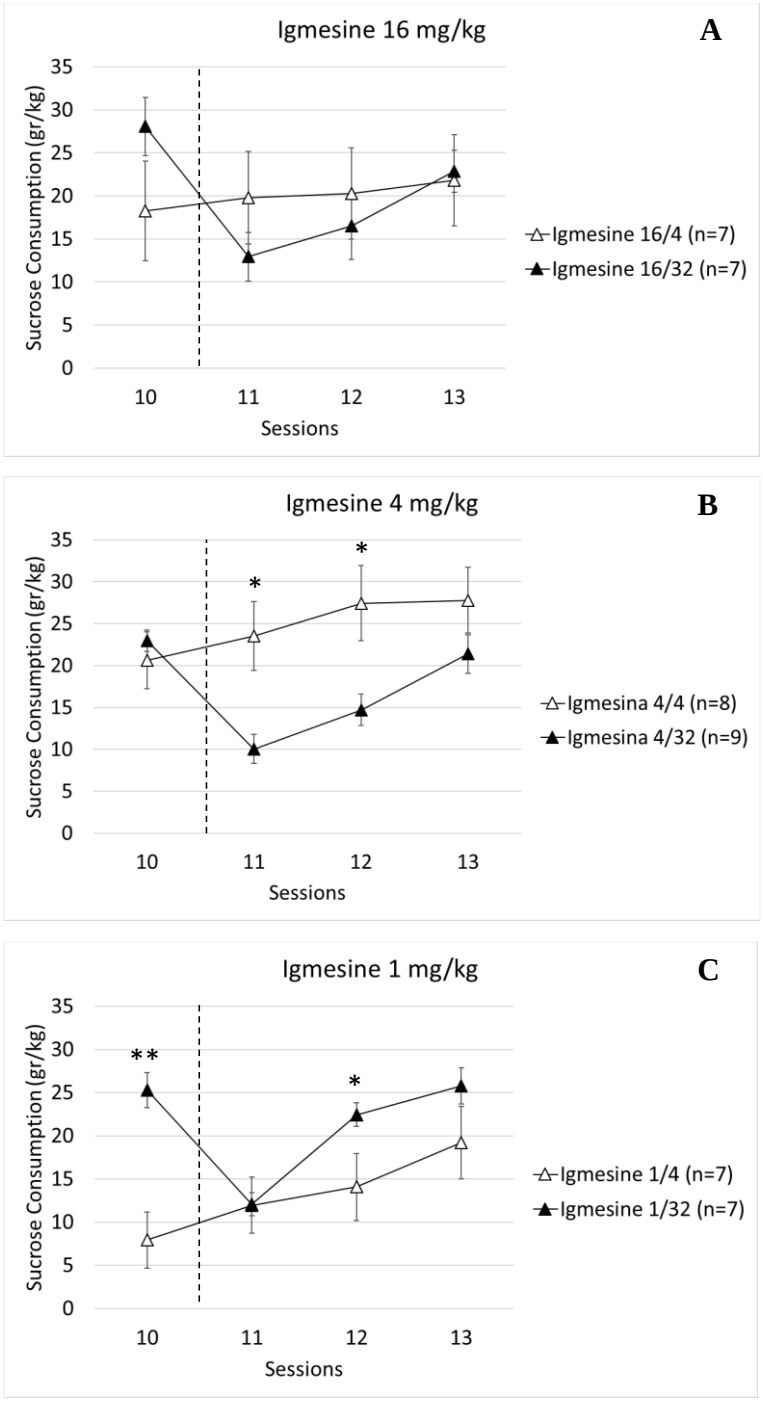

Figure 23. Igmesine administration in Consummatory Successive Negative Contrast. Figure shows MEAN (± SEM) of sucrose consumption in the cSNC protocol after Igmesine administration. Drug dose (16, 4 y 1 mg/kg)/32: animals exposed to reward devaluation from 32% to 4% sucrose during postshift sessions. Figure showed MEAN ± SEM of sucrose consumption in the cSNC protocol after Igmesine Sig1R agonist administration. (A) The effect of Igmesine 16 mg showed no differences between groups during postshift phase. The administration of this dose eliminated the frustration. (B) The effect of Igmesine 4 mg showed a frustration that lasted two days. (C) The effect of Igmesine 1 mg/kg showed an elimination of frustration during the first postshift session. Results suggest an "U" shape doses-dependent response. The dashed line separates the preshift (day 10) and postshift (days 11–13) sessions. Statistically significant differences between the animals exposed to a high (32 %) and low (4 %) reinforcer: *p. <0.05 and ** p. <0.01

Depending on the Sig1R agonist administered, a different response has been observed. Both PRE-084 and igmesine seem to eliminate or reduce the contrast.

However, igmesine has shown an "U" shape dose-response similar to the patter elicited after the administration of BD1063. DER for the Sig1R agonists in the cSNC is represented in Figure 24.

Contrary to our expectations, DER indicated a better effectivity of igmesine 16 mg/kg in comparison with the other tested doses with this drug. On the other hand, the DER evidenced a great effectivity of PRE-084 with 8 mg/kg in cSNC procedure. According to Flaherty's analyses, only CDP and ethanol have obtained such high level of effectivity. On the other hand, the effectivity found for PRE-084 2 mg/kg was similar to that found with morphine and flurazepam (Flaherty, 1996). Both statistical and DER analyses have shown that agonists of Sig1R seem to modulate the processes involves in frustration.

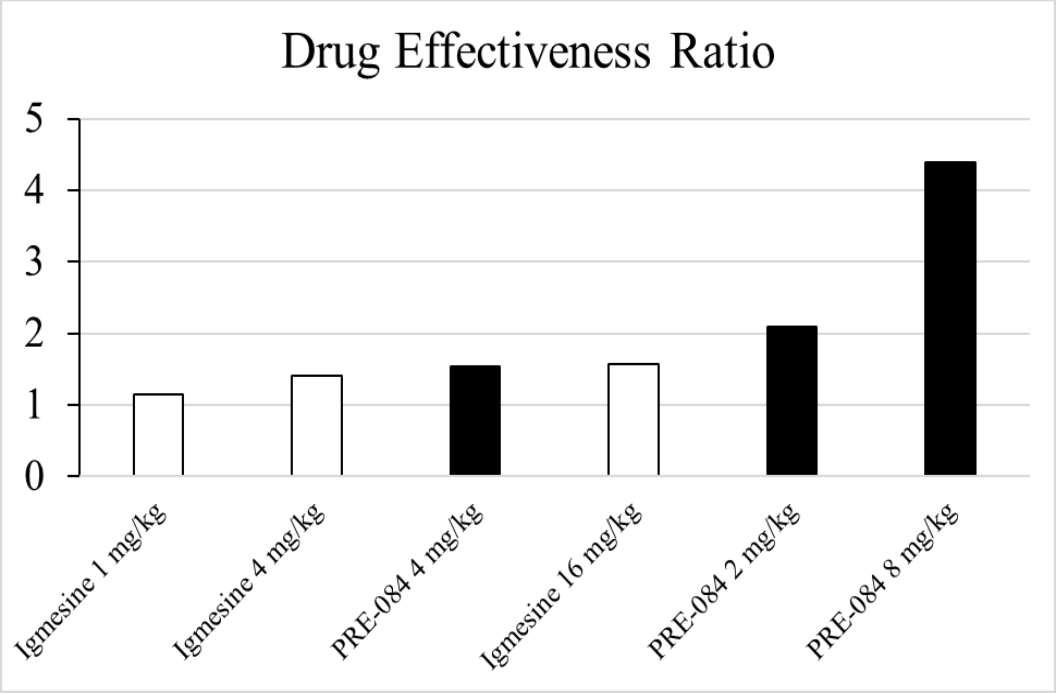

Figure 24. Drug Effectiveness Ratio of Sig1R agonists igmesine and PRE-084. DER shows the efficacy of agonist drugs in increasing order. PRE-084 8 mg and 2 mg have indicated an elimination of frustration, whereas igmesine 1 mg/kg evoked a reduction of frustration in the first postshift session.

3.3.Discussion of the effects of sigma-1 ligands

A summary of the main results obtained with the administration of Sig1R ligands are represented in Table 8 and it will be discussed below.

The two main aspects in this experiment that need to be discussed: (1) the differences between the control and the experimental group in the preshift and postshift sessions, and (2) the comparison of the effects induced by the Sig1R ligands in the cSNC.

Table 8. **Summary of the results found in Experiment 3.** Results have been identified depending on the differences found in two independent samples comparisons and the DER value (a score higher to 1.5 has been considered as effective). It has been represented the drug profile (Sig1R agonist or antagonist), names, the doses administrated, and the main effects found in the comparison between groups. ++ strong effect; +/-: moderate effect; -: no effect

Profile	Drug	Doses (mg/kg)	Postshift 1
Antagonist	**S1RA**	64	-
		32	-
		16	-
	BD1063	16	+
		4	-
		1	+/-
Agonist	**PRE-084**	8	++
		4	++
		2	++
	Igmesine	16	++
		4	-
		1	+/-

The effects of different drugs in cSNC have been widely studied. However, the SNC needs to obtain significant differences between the experimental group and the control group in the preshift phase. The vehicle administration has exhibited this difference, i.e., the experimental group (exposed to 32 % sucrose) has drank more sucrose than the control group during the preshift phase. This result is evoked due to the higher reinforcement (32 % sucrose) it is considered more reinforcing than the lower

reinforcer (4 % sucrose) (Flaherty, 1996). However, the preshift phase values obtained on day 10 in some experimental groups (exposed to the high reinforcer), such as S1RA 16 mg/kg, BD1063 4 mg/kg, PRE-084 8 and 2 mg/kg and igmesine 4 mg/kg, did not reach a notable difference (or no differences) in comparison with the consummatory values obtained in the control group (low reinforcer; 4% sucrose). As it has been mentioned before, this parameter is essential to induce the frustration contrast. Furthermore, there was also a considerable variability on the postshift 1 (i.e., session 11) consummatory values in various control groups. There-fore, a large number of the behavioral responses observed on preshift and postshift phases were influenced by this variability. For instance, when we compare the effects of PRE-084 between preshift and postshift 1 in the control group, two different patterns of sucrose consumption was observed: a great reduction and a moderate increment, for 8 mg/kg and 4 mg/kg of PRE-084, respectively (Figure 21). In addition, these differences of the consumption have significantly influenced our results since the statistical analyses have considered the changes between preshift and postshift sessions. This circumstance can be clearly identified analyzing the above-mentioned data. According to the statistical analysis performed after the administration of PRE-084 8 mg/kg and 4 mg/kg, both doses eliminated the frustration on postshift 1, but the differences on the reduction of sucrose consumption were conditioned by the control group baseline (Figure 21). Similar results regarding the preshift baseline and postshift values can be found with BD1063 16 mg/kg and 4 mg/kg (Figure 19). Thus, our results after the administration of the Sig1R ligands might be strongly conditioned by these crucial experimental aspects of the cSNC.

Some consequences according to the paradigm used (cSNC versus iSNC) might have affected these results. cSNC has demonstrated a different cerebral activity compared to iSNC (Adelman & Maatsch, 1956; Amsel, 1967; Flaherty, 1996; Pellegrini et al., 2004; Pellegrini & Papini, 2007). In particular, it has been sup-posed, for SNC procedure, and entorhinal – hippocampal- cingulate- striatal system to the instrumental contrast and the solitary tract- PBN-Hypothalamus and limbic system – amygdala to the consummatory contrast. The passive behavioral performance of the cSNC procedure involves a pathway with a great modulation of limbic system and the parabraquial nuclei which is related to the pleasure associated to the reinforcer while iSNC procedure involves a pathway that includes memory, reinforcement and learning areas. For this

reason, it is possible, that the lack of activation of some frustration processes (memory or learning mainly) in cSNC might be caused by the variability of Sig1R agonists activity found in Experiment 3 or the mitigated effect which has been found in postshift 1 from the administration of morphine (Rowan & Flaherty, 1987) or DPDPE (Wood et al., 2014), among others. Hence, according to Papini (2009), there is an allocentric process (knowledge of the environment and the adjustment of the behavior with respect to this environment by the subject) that is suggested to appear in postshift 1. It is possible that some of these animals did not learn the task owing to the very nature of the cSNC paradigm (and the activated areas) or the complexity of frustration that might have interfered in the results.

Nevertheless, we tried to evaluate the pharmacological effects of the drugs under study using a different analytical approach as previously was described, the Drug Effectiveness Ratio (DER). The application of the DER formula designed by Flaherty (1996) eliminated those differences (between preshift day 10 and postshift day 11) and it normalize the consumption of control and experimental groups (see section 2.2 Material and Methods). Therefore, we analyzed the efficacy of Sig1R ligands using this calculating approach (Figures 20 and 23). The summary of the results produced by the Sig1R ligands in relation to their DER score is presented in Table 8. Considering all the methodological and analytical issues remarked above, the results of the Sig1R ligands in the cSNC paradigm will be discussed taking into account the DER results.

Flaherty and coworkers (1980) found a reduction of frustration, on the first postshift day, after the repeated administration of benzodiazepines during several days before the contrast (Flaherty et al. 1980). According to the DER classification, the drug with the best effectiveness was the agonist PRE-084, because a dose of 8 mg/kg induced a total elimination of frustration acting even on the first postshift day (Figures 22 and 24). In comparison with some results found by Flaherty, PRE-084 8 mg/kg showed a similar effectiveness to that induced by chlordiazepoxide plus ethanol (Becker & Flaher-ty, 1983). It is important to highlight that the administration of some Sig1R ligands yielded a reduction of the first postsession frustration even after only one administration 30 minutes before the procedure. The injection of the Sig1R agonist igmesine also seems to reduce the contrast (Figure 22 and Figure 23). As mentioned before, both agonists PRE-084 and igmesine have mitigated or abolished the contrast in our studies. As previously mentioned, Sig1R agonists have been found to modulate

depression, anxiety, learning and memory impairment in different pharmacological and pathological models in rodents (reviewed by Cobos et al., 2008; Maurice and Goguadze, 2017). In particular, igmesine and PRE-084 have been shown to reduce the depression and improve the memory impairment with a single dose (Matsuno et al., 1996; Urani et al., 2001). Our data shows that PRE-084 (at all doses) has been the only drug which fully eliminated the frustration, whereas igmesine also reduces the frustration at doses of 16 mg/kg and 1 mg/kg. The latter has been the only dose which recovered the consumption during the postshift to the levels indicated in preshift phase (Figure 22). Therefore, all these evidences suggest a possible role for the Sig1R agonists to modulate the frustration processes.

On the other hand, as mentioned in the Introduction section, a relationship between pain and frustration have been demonstrated (Mustaca and Papini, 2005; Ortega et al., 2011). In particular, a lower sensitivity to pain or hypoalgesia has been found after a cSNC paradigm application (reviewed by Papini et al., 2015). In fact, the results of our experiment 2 support these previous findings related to the hypoalgesia induced by frustration processes. In addition, there is an anatomical and physiological convergence between pain, negative effect and motivation known as adaptive control hypothesis (Shackman et al., 2011). The role of Sig1R antagonist reducing o abolishing different pain conditions has been supported by numerous studies (Zamanillo et al., 2013; Merlos et al., 2017). When we evaluated the actions of BD1063 and S1RA in the cSNC, contradictory results were observed. The s.c. administration of the Sig1R antagonist BD1063 at doses of 16 mg/kg and 1 mg/kg induced a reduction of frustration, supporting the adaptive control hypothesis (Shackman et al., 2011). In contrast, the administration of the selective Sig1R antagonist S1RA did not modify the frustration response at any of the tested doses (Figure 18). This apparent discrepancy may be related to the lack of preshift differences between the experimental and control groups, as we discussed above. Moreover, the administration of PRE-084 has shown to enhance the pain perception in several pain models (Roh et al., 2008; Entrena et al., 2016) but the administration of this drug did not increase the frustration. Besides the incapacity of S1RA to reduce or eliminate the frustration on the first postshift day may indicate that there is no physical pain on the first day. Thus, the psychological pain (related to frustration processes) described by Papini and colleagues (2015) in terms of “an aversive state induced by actual or anticipated reward omission or devaluation in a

nonsocial context" suggest an-other type of negative effect that does not involve physical pain (Papini et al., 2015). Therefore, these antecedents also support that the effects of the PRE-084 and igmesine on the frustration may be related to their anxiolytic actions instead of their pain-related effects.

Our pharmacological results obtained administering Sig1R ligands suggest that frustration, induced by cSNC, does not involve a physical pain but the modulation of anxiety, memory and learning. The great effectiveness of benzodiazepines to reduce the frustration on the second postshift day support the appearance of anxiety through repeated exposure to the frustrating cue. However, it is suggesting the existence of other processes which requires memory and learning on the first postshift day. The results found in the Experiment 1 suggest that it is possible that the animal is not completely aware of the devaluation pointing out a "shock" outcome.

In summary, we found that the Sig1R agonists seems to mitigate the frustration in the cSNC paradigm, whereas the actions of the Sig1R antagonists were conflicting. However, due to the great variability observed during the cSNC procedure, further studies to support our data adding more Sig1R ligands or testing Sig1R knockout animals are needed.

Conclusions

1. Specific conclusions

1. Experimental manipulation of the reinforcer (number of pellets) during the pre-shift phase in the iSNC paradigm, by gradually increasing the reinforcer, a weak contrast effect is produced in comparison to the experimental group, which yields a 3-days frustration post-shift period.
2. The frustration induced by the reward devaluation in the cSNC paradigm induces a decrease in the pain sensitivity (i.e., hypoalgesia) evoked by a mechanical pain stimulus (von Frey test) and a heat pain stimulus (Hargreaves test).
3. Frustration causes a hypoalgesia that continues immediately after the devaluation and lasts up to five hours 5 hours, only on the second day of contrast, independently of the pain stimulus (mechanical or heat) applied.
4. The systemic administration of Sig1R ligands differentially modulate the frustration induced by the cSNC. The differential actions observed seem, in some cases, to be mediated by the lack of differences between the control and the experimental groups in the pre-shift and post-shift sessions of cSNC.
5. The subcutaneous administration of the selective Sig1R agonists (PRE-084 and igmesine) reduces or eliminates the frustration both on the first and on the second post-shift days.
6. The subcutaneous administration of the selective Sig1R antagonists produce controversial results, because BD1063 seems to reduce the frustration whereas S1RA do not modify the frustration response at any of the tested doses.

2. General conclusions

Frustration can be mitigated if there has been previous experiences with similar low levels of reinforcement.

SNC has demonstrated to be a very complex model. From the first to the second postshift day, the behavioral response has been attenuated. From the physiological evaluation carried out, the response recorded tends to remain over the days. That physiological response is maintained on the second postshift day, 300 minutes after the devaluation of the enhancer.

Frustration involves processes such as memory, learning and anxiety. The reduction of frustration in post-shift 1 and 2 through a single administration of Sig1R agonist drugs supports this hypothesis. However the effects of the Sig1R antagonist drugs are controversial.

Resumen

1. Antecedentes, hipótesis y objetivos

1.1. Antecedentes

Actualmente, 9 de cada 10 ciudadanos españoles informan haber sufrido estrés en el último año (Maset, 2017). Se estima que los tipos más habituales de estrés engloban trastornos como la ansiedad o la depresión, los cuales son cada vez más frecuentes en la población (Gancedo-García et al., 2019). Un trauma infantil, la personalidad, la muerte de un pariente, un divorcio o problemas económicos son algunos ejemplos de situaciones que pueden desencadenar un trastorno de ansiedad o del estado de ánimo (Kamenetzky et al., 2009). Algunos autores sugieren que aquellas situaciones estresantes que son provocadas por una reducción o devaluación del reforzador (por ejemplo, un despido o divorcio) se denomina frustración (Amsel, 1992). Habitualmente, se puede observar como la respuesta conductual es diferente entre individuos, existiendo una clara variabilidad dependiendo de variables contextuales (el entorno en el que ocurre la devaluación) (Sánchez et al., 2001) y la experiencia previa del bajo reforzador (haber sufrido otras experiencias similares anteriores de devaluación) (Flaherty et al., 1983; Shackman et al., 2011). Esta pérdida o devaluación inesperada de la recompensa desencadena una serie de cambios comportamentales (agresión e ira), fisiológicos (hormonales e inmunológicos) y cognitivos de tipo aversivo que se condicionan la respuesta de frustración (Amsel, 1992; Papini, 2006). Es por toda esta variabilidad, que se ha considerado la frustración como un estado emocional objeto de estudio en numerosos campos. Además, como ya se ha mencionado, esta respuesta de frustración presenta cierta variabilidad conductual asociada a las experiencias previas. Un claro ejemplo de esto son los resultados obtenidos en experimentos de Contraste Sucesivo Negativo (CSN) en los que el animal recibe una devaluación u omisión de un reforzador apetitivo esperado y comparado con un grupo control que recibe siempre el reforzador devaluado (Papini, 2006). La manipulación experimental de la fase previa a la devaluación mediante eliminación intermitente del reforzador (reforzamiento parcial) o el incremento gradual del reforzador provocan modificaciones en la respuesta de frustración del animal, atenuando en ambos casos a la misma (Amsel, 1967; Cuenya et al., 2012; Flaherty et al., 1983). Por lo tanto, la frustración es una respuesta compleja sensible a experiencias previas en la fase anteriro a la devaluación del refuerzo.

La frustración, a su vez, es un proceso emocional estudiado ampliamente. El interés de algunos autores ha sido el de estudiar las interacciones con otros procesos neurobiológicos como el dolor, la ansiedad o la depresión (Kameneztky et al., 2009). En relación con la percepción del dolor, existen una gran cantidad de similitudes con la frustración a un nivel neurobiológico, farmacológico y de procedimiento (Papini et al., 2015). Sin embargo, poco se ha estudiado sobre su interacción. Algunos estudios encontraron que la frustración (inducida mediante el Contraste Sucesivo Negativo o CSN) produce hipoalgesia (medida a través el test de la placa caliente) pero solo en el segundo día de exposición a la pérdida de recompensa o devaluación (Mustaca y Papini, 2005). En la misma línea, en un experimento en el que se aplicaba formalina en la pata trasera antes de la devaluación, con la finalidad de inducir dolor físico, se encontró que este tipo de dolor inflamatorio potenciaba la frustración (Ortega et al., 2011). En ambos experimentos se utilizó un estímulo térmico o uno químico, pero en ninguno se estudió la interacción entre el dolor físico y la frustración mediante un estímulo mecánico. Numerosos estudios han hallado que el estrés activa mecanismos analgésicos tanto opioides como no opioides (Papini et al., 2015). El estudio de la interacción entre la frustración y el dolor físico, así como su respuesta tras varias horas desde la exposición al estímulo forman parte del objetivo a cumplir en esta Tesis Doctoral.

Uno de los campos más interesados en el estudio de la frustración ha sido el de la farmacología. Esto se ha debido principalmente a que los fármacos comercializados para la depresión y la ansiedad requieren en muchas ocasiones un tiempo mínimo de 2 semanas para que se puedan observar sus efectos y, además, en numerosas ocasiones inducen una gran cantidad de efectos secundarios tales como tolerancia, dependencia y ansiedad tras un uso continuado (Hayashi, 2015). Por lo tanto, dichos fármacos no llegan a satisfacer las necesidades de los pacientes (Tottori et al., 2001). Estudios previos han señalado que la frustración modula procesos como la memoria (Bentosela et al., 2006), el aprendizaje (Wood et al., 2005), el dolor (Mustaca y Papini, 2005) y el miedo o la ansiedad (Flaherty et al., 1998) siendo, por lo tanto, el objetivo para la administración de benzodiacepinas, opiáceos y antidepresivos, entre otros (Bentosela et al. 2006, Flaherty, 1996; Wood et al. 2005).

En la búsqueda de nuevos fármacos para el tratamiento de la frustración una de las posibles dianas terapéuticas se ha enfocado hacia aquellos fármacos que actúan sobre los receptores sigma-1 (Tottori et al., 2001;Cobos et al., 2008; Merlos et al., 2017).

Existen evidencias de que los receptores sigma-1 parecen actuar regulando varios procesos en el sistema nervioso central tales como el dolor, aprendizaje, la memoria y la neuroprotección (Turk et al., 2011; Zamanillo et al., 2013), procesos en los que se había señalado el papel de la frustración.

El receptor sigma-1 (Sig1R) es una chaperona intracelular compuesta por 223 aminoácidos que no guarda relación estructural con ninguna otra proteína conocida en los mamíferos, y cuya estructura sigue generando controversia en cuanto a si posee uno o dos dominios transmembrana (Hayashi, 2019). El Sig1R tiene una rica farmacología siendo los antagonistas selectivos mejor estudiados el NE-100, BD-1047, BD-1063 y S1RA, mientras que el agonista más utilizado es el PRE-084 (Cobos et al., 2008; Romero et al. 2012). La implicación de estos receptores en motivación, aprendizaje y memoria, así como su distribución por el sistema límbico y el tronco cerebral, han llevado a proponer al Sig1R como una nueva diana terapéutica (Sabino et al., 2009, Chu & Ruoho, 2016).

La investigación llevada a cabo en modelos animales sobre los trastornos de ansiedad o estrés se han considerado modelos poco traslacionales o clínicos (Kameneztky et al., 2009). Por lo tanto, se ha sugerido el Contraste Sucesivo Negativo o CSN como un modelo animal muy útil en el que las expectativas o interpretaciones del animal en sí mismo son causa de frustración. El procedimiento del CSN presenta dos modalidades; consumatorio o CSNc e instrumental o CSNi. Así, ambos procedimientos precisan de una devaluación en la calidad (para el caso del consumatorio) o de la cantidad (para el caso del instrumental) del reforzador esperado. Del mismo modo, el CSN ha demostrado ser un modelo sensible a fármacos ansiolíticos y opioides (Flaherty, 1980; Ortega et al., 2013) siendo un modelo animal viable y traslacional para el estudio de nuevos fármacos, entre los que se encontrarían los ligandos que actúan sobre los Sig1R.

1.2. Hipótesis y objetivos

El objetivo principal de esta Tesis Doctoral ha sido estudiar el proceso de frustración inducido por el paradigma de la devaluación recompensas denominado Contraste Sucesivo Negativo (CSN). Para estudiar este paradigma se han utilizado enfoques conductuales, fisiológicos y farmacológicos.

Basado en la sensibilidad de la frustración a las experiencias previas a la devaluación del refuerzo (Flaherty et al., 1983), se presume que la manipulación experimental en la fase precambio del CSN causa diferentes respuestas de comportamiento asociadas con esa experiencia previa. Flaherty y col. (1983) encuentran una expresión mitigada de frustración después de un aumento gradual del reforzador en el paradigma consumatorio del CSN. Las diferencias neurobiológicas entre los procedimientos consumatorio e instrumental sugieren que la respuesta entre estos dos tipos de CSN puede ser diferente (Flaherty, 1996; Pellegrini y Papini, 2007).

Por lo tanto, también se plantea la hipótesis de que el grupo con una experiencia previa de alto refuerzo presentará una mayor frustración cuando se devalúe su refuerzo en comparación con el grupo de control que siempre recibirá el refuerzo bajo. Del mismo modo, se presume que el nivel de frustración será mayor en el grupo experimental en comparación con el grupo de incremento que recibirá un reforzador *in crescendo* durante la fase precambio en el procedimiento instrumental de SNC (**Hipótesis 1**).

Para probar esta hipótesis, el *primer objetivo* ha sido evaluar las diferencias de comportamiento derivadas de la manipulación de expectativas en la devaluación de las recompensas del paradigma CSNi. Por este motivo, se han evaluado 3 grupos: un grupo de control, un grupo experimental y un grupo de incremento. El grupo experimental ha tenido acceso a 12 *pellets* durante la fase precambio, mientras que el grupo control ha tenido acceso a 2 *pellets*. El grupo de incremento tuvo un aumento gradual durante las sesiones (2, 4, 6, 8, 10 y 12 *pellets*). Posteriormente, todos los animales tuvieron acceso a 2 *pellets* en la fase postcambio. Para registrar las diferencias de comportamiento asociadas con la manipulación experimental, se registró el tiempo que tardaron los animales en atravesar el laberinto y alcanzar el reforzador esperado.

Estudios previos han indicado la existencia de una correlación neurobiológica y farmacológica entre el dolor físico y los procesos de estrés o ansiedad (Torres et al., 2015). Se ha sugerido que una respuesta de ansiedad inducida por una devaluación de la recompensa puede modular el dolor físico (Mustaca y Papini, 2005). En ese sentido, se espera que los animales que hayan sido expuestos a una experiencia previa de frustración presenten una mayor respuesta hipoalgésica, es decir, una disminución de la sensibilidad y un aumento en el umbral frente a estímulos dolorosos, en comparación a los animales que no han sido frustrados (**Hipótesis 2**).

Para probar esta hipótesis, el *segundo objetivo* ha sido estudiar las diferencias en la percepción del dolor (medido a través de las pruebas de von Frey y Hargreaves) después de una experiencia previa de frustración inducida por el CSNc. Por otro lado, el *tercer objetivo* ha sido estudiar las respuestas conductuales referidas a la evaluación de dicha percepción. Esto se llevó a cabo durante el primer y segundo día de la fase postcambio (es decir, el primer y segundo día de devaluación) inmediatamente después y 300 minutos después de la devaluación.

Finalmente, la evidencia farmacológica previa señaló a Sig1R como un posible modulador de los procesos asociados con las experiencias de frustración. El antagonismo Sig1R ha demostrado ser efectivo contra el dolor (Zamanillo et al., 2013; Merlos et al., 2017), mientras que los agonistas Sig1R han demostrado un efecto ansiolítico y una mejora en el aprendizaje y la memoria (Urani et al., 2001; Maurice y Goguadze, 2017). Por ese motivo, se presume que Sig1R podría modular los procesos asociados con la frustración (**Hipótesis 3**).

Nuestro *cuarto objetivo* ha sido evaluar los efectos de los ligandos Sig1R sobre la frustración. Para lograr este objetivo, se han administrado los antagonistas selectivos de Sig1R BD-1063 y S1RA y los agonistas selectivos de Sig1R PRE-084 e igmesina en el primer día postcambio del paradigma CSNc. Se espera que los antagonistas de Sig1R actúen reduciendo el dolor derivado del proceso de frustración, mientras que los agonistas de Sig1R podrían mitigar la ansiedad inducida por la frustración.

2. Materiales y métodos

2.1. Animales de experimentación

Se utilizaron ratas Wistar macho obtenidas de los laboratorios de Harlan (Barcelona, España), Envigo (Barcelona, España) y Charles Rivers (Les Oncins, Francia). Los animales tenían aproximadamente entre 75-90 días de edad al comienzo de cada experimento.

Las ratas se alojaron individualmente en jaulas de policarbonato con agua *ad libitum*, en una habitación con temperatura (22 ° C) y humedad (50-60%) constante. Los animales fueron alojados bajo un ciclo de luz: oscuridad de 12:12 h (las luces se encendían a las 08:00 h) y privados al 82-85% de su peso ad libitum durante todo el

experimento. El protocolo experimental fue aprobado por el Comité de Ética de Investigación de la Universidad de Granada (20-08-15-294 y 09/08/2019/134).

2.2. Modelos animales para inducir frustración

El modelo animal llevado a cabo para inducir la frustración ha sido el Contraste Sucesivo Negativo. Dicho procedimiento compara un grupo experimental con un grupo control. El grupo experimental recibe una experiencia previa de alto reforzador durante una fase precambio, el cual es devaluado durante la fase postcambio a un reforzador de menor cantidad y/o calidad. Por otro lado, el grupo control recibe el bajo reforzador durante todo el procedimiento. Así, dicho procedimiento presenta dos modalidades: instrumental y consumatorio, que se expondrán a continuación.

2.2.1. Contraste Sucesivo Negativo Instrumental

El CSNi requiere de un instrumento por el que el animal tiene que aprender una tarea y sobre la cual se obtendrá el reforzador correspondiente. Dicho procedimiento se llevó a cabo durante el Experimento 1. El instrumento utilizado ha sido un laberinto recto de dimensiones 120 (largo) x 14 (alto) x 11 (ancho) cm con un área de salida de 20 cm, un área de meta de 20 cm y un área central de 80 cm. Del mismo modo, en el experimento el reforzador consistió en bolitas azucaradas denominados *pellets*. La variable dependiente registrada en este procedimiento fue el tiempo en recorrer el laberinto, el cual se midió con un cronómetro.

En el procedimiento del CSNi, los animales tienen que correr a través de un laberinto recto para recibir los *pellets*. El alto reforzador (12 *pellets*) se ha administrado en 6 ensayos diarios durante 10 días, seguido de 4 días con 6 ensayos diarios de acceso al bajo reforzador (2 *pellets*). El efecto de frustración en el CSNi implica una supresión en el tiempo en recorrer el laberinto después de la devaluación de 12 a 2 *pellets*, seguido de una recuperación al nivel de los controles no desplazados en los días posteriores.

El procedimiento utilizado en el Experimento 1 fue similar al descrito previamente por Flaherty et al. (1987), por el que se añadió otro grupo (grupo de incremento) que recibió una experiencia previa *in crescendo* a lo largo de la fase precambio.

En el primer día de la fase de habituación se realizaron 5 ensayos con cada rata independientemente del grupo al que perteneciera (entre ensayo y ensayo había unos 20

minutos). Cada ensayo consistía en 1 minuto de recorrido libre por el laberinto sin *pellets*. Durante el segundo día de habituación se realizaron un total de cinco ensayos que podrían ser de tres tipos: El primer tipo incluyó dos ensayos de 1 minuto de duración cada uno en los que la rata hacia recorrido libre por el laberinto sin *pellets*. Tras el primer tipo de ensayo se realizó el segundo tipo, en donde se dejaba a las ratas encerradas en la caja meta con 12 o con 2 *pellets* dependiendo del grupo experimental al que pertenecieran. El tiempo máximo de duración fue de 1 minuto. Tras el segundo tipo, se realizó un último ensayo en el que se colocaban los *pellets* (12 ó 2) a lo largo del laberinto y se exponía a la rata al mismo durante 1 minuto. Durante el último día de habituación se realizaban 3 ensayos con cada rata en los que se dejaba a las ratas encerradas en la caja meta con 12 ó 2 *pellets* dependiendo del grupo experimental al que perteneciera. Si la rata no se comía los *pellets* en esta fase, se dejaba 30 segundos más para después sacarla independientemente de si se los hubiera comido o no. Además, en el último día de la fase de precambio se incluyeron 12 *pellets* con la comida habitual en la jaula hogar para todas las ratas.

A continuación, se realizó la fase de precambio con una duración de 6 días. En esta fase, se realizaban 6 ensayos diarios con 20 minutos aproximadamente entre ellos. En primer lugar, cada ensayo empezaba con la colocación de cada rata en la caja de salida con la puerta cerrada. Tras abrirse la puerta, se permitía a la rata recorrer el laberinto durante un máximo de 20 segundos, registrando el tiempo que tardaba. Sin embargo, si transcurrían los 20 segundos sin llegar a la meta, se le empujaba hasta ella y se registraban 20 segundos. Cuando llegaba a la caja meta, se cerraba la puerta y se dejaba un máximo de 30 segundos para el consumo de *pellets*. El número de *pellets* que había en la caja meta dependía del grupo experimental al que pertenecía cada rata. Para el grupo 2-2 había 2 *pellets*, para el grupo 12-2 había 12 *pellets*, mientras que para el grupo 2,4,6,8,10,12-2 el número de *pellets* para el primer día de precambio fue de 2, incrementándose en dos *pellets* cada día hasta llegar al último día con un total de 12 *pellets*. Finalmente, se sacaba a la rata y se limpiaba el laberinto cuando era necesario

El último paso de este paradigma consistió en implementar la fase de postcambio durante 4 días. Esta fase contaba con 6 ensayos al día con unos 20 minutos aproximadamente entre cada ensayo. El procedimiento realizado fue el mismo que en la fase precambio, pero en este caso todos los animales recibieron 2 *pellets* en la caja meta independientemente del grupo al que pertenecían.

2.2.2. Contraste Sucesivo Negativo Consumatorio

El procedimiento CSNc se realizó en una caja de plexiglás de 15 cm x 30 cm x 30 cm. La variable dependiente registrada en este procedimiento es el consumo de sacarosa, el cual se registró a través de buretas graduadas a 0,01 mililitros (ml) en el Experimento 2 y de gramos por kilo (g/kg) en el Experimento 3.

La sacarosa se preparó al peso. Así, para la sacarosa al 32%, se pesaron 32 gramos de azúcar comercial y 68 gramos de agua, mientras que para la sacarosa al 4% se pesaron 4 gramos de azúcar comercial y 96 gramos de agua.

En el procedimiento CSNc los animales pertenecientes al grupo experimental tienen acceso libre durante 5 minutos a una solución de sacarosa con alto valor reforzante (32% de sacarosa) durante varias sesiones diarias, comparado con animales del grupo control que han recibido acceso a una solución con bajo reforzador (4% de sacarosa). Durante la fase postcambio ambos grupos (experimental o devaluado y el grupo control) pasan a recibir el reforzador de bajo valor, es decir, el 4% de sacarosa. Así, durante la presente Tesis Doctoral se mencionará el grupo control como grupo que siempre recibe un bajo reforzador y el grupo experimental como grupo que recibe una devaluación del reforzador.

La frustración expresada en el procedimiento CSNc implica una supresión del comportamiento de consumo (es decir, una reducción en la cantidad de sacarosa consumida) después de la devaluación de sacarosa del 32 al 4%, seguido de una recuperación al nivel de los controles al paso de los días.

No existe un tiempo establecido para que se provoque la frustración. Por este motivo, los días de la fase precambio varían en función del experimento realizado, siendo de 16 días para el Experimento 2 y de 10 días para el Experimento 3. Posteriormente, la fase postcambio requiere un tiempo mínimo de 3 días para registrar la frustración y posterior recuperación al nivel de los controles. La fase postcambio duró 3 días en el Experimento 2 y 5 en el Experimento 3. Independientemente de estas manipulaciones, ambos grupos mostraron frustración asociada a la devaluación del reforzador.

Por otro lado, durante el Experimento 2 los animales fueron divididos en aquellos que iban a ser evaluados en la prueba de von Frey o en el test Hargreaves durante el

primer o segundo día postcambio. En el Experimento 2 se establecieron un total de 8 grupos distribuidos de la siguiente forma: 2 grupos experimentales (1 de ellos evaluado el primer día y otro el segundo díade la fase postcambio) y 2 grupos control (1 de ellos evaluado el primer día y otro el segundo díade la fase postcambio) para cada uno de los procedimientos de evaluación de la percepción del umbral doloroso (von Frey y Hargreaves).

Durante el Experimento 3, se establecen 2 grupos, uno experimental y otro control para cada uno de los fármacos administrados, incluido el suero fisiológico.

Ratio de Efectividad de la Droga

Flaherty (1996) diseñó una fórmula específica para el CSNc y para medir la efectividad de los fármacos en la reducción de la frustración (para más información, ver Figura 10). Esa fórmula compara la proporción de consumo del grupo frustrado en comparación con el grupo control durante el último día precambio y el primer día postcambio. Por lo tanto, esa fórmula indica cómo se ha reducido el consumo en el grupo frustrado en comparación con el grupo control y, asimismo, comparado con el vehículo, es decir, con la administración de solución salina.

Una gran reducción del consumo de sacarosa indica que el animal se ha frustrado y, por lo tanto, que el fármaco tiene una baja eficacia al reducir ésta. La fórmula indicaría una puntuación cercana a 0 en este caso. Por otro lado, una alta eficacia indica una pobre reducción del consumo, indicando puntuaciones superiores. Por ejemplo, fármacos eficaces como el Clordiacepóxido (CDP) dan puntuaciones cercanas al 3,6 mientras que fármacos ineficaces como la anfetamina dan puntuaciones de 1,1 (Flaherty, 1996).

La fórmula original diseñada por Flaherty (1996) consideró la frecuencia del lameteo de la boquilla de la botella, debido a que esa era la variable independiente registrada inicialmente en el procedimiento. Sin embargo, en el Experimento 3 se han utilizado los gr/kg tal y como se ha comentado anteriormente.

2.3. Modelos animales para medir la percepción del dolor

En el Experimento 2, se utilizaron el test de von Frey (Chaplan et al., 1994) y el test de Hargreaves (Hargreaves et al., 1988) para detectar los umbrales dolorosos frente a estímulos mecánicos y térmicos, respectivamente.

2.3.1. Test de von Frey

La aplicación de los filamentos de von Frey es una prueba de evaluación no invasiva de los niveles de percepción sensorial de la piel en todo el cuerpo con resultados objetivos y replicables. Cada filamento se calibra individualmente para ofrecer su resistencia específica dentro de una desviación estándar del 5%.

La evaluación con el test de von Frey se llevó a cabo inmediatamente y 300 minutos después de la primera y la segunda sesión postcambio. La mitad de los animales fue evaluada el primer día y la otra mitad el segundo día de la fase postcambio. Por otro lado, y para minimizar el efecto de novedad a las cajas de von Frey, los animales fueron habituados en el procedimiento del CSN en días alternos durante 5 minutos. Estas sesiones de habituación se llevaron a cabo durante las sesiones precambio. Además, antes del protocolo CSNc, los animales fueron evaluados para determinar una línea de base previa a cualquier manipulación experimental.

Cada evaluación siguió el método *arriba-abajo o up-down* y consistió en la aplicación de los filamentos un mínimo de 6 veces y un máximo de 15 veces. Cada filamento fue aplicado tres veces durante 2-3 s, separados por intervalos de 5 s (Chaplan et al., 1994). Las pruebas comenzaron con el filamento de von Frey de 2 g (19,6 mN) que se encuentra en la mitad del rango. La pata elegida para la estimulación siempre fue la misma para un animal determinado, siendo la mitad de los animales de cada grupo evaluados en la pata trasera izquierda y la otra mitad en la pata trasera derecha. Si en cada prueba con un filamento determinado no había respuesta, se seleccionaba uno de mayor fuerza. Si, por el contrario, el animal respondía, se utiliza un filamento de menor fuerza en el siguiente ensayo. Se consideraba que el animal había respondido al estímulo cuando ocurría una retirada inmediata de la pata. Se realizó una técnica de simple ciego con respecto a la asignación de contraste del sujeto (es decir, 32% frente a 4% de sacarosa).

La variable registrada por el test de von Frey determina la fuerza (expresada en gramos) mínima en la que el animal respondería en el 50% de las ocasiones que fuera evaluado con el mismo.

2.3.2. Test de Hargreaves

La prueba de Hargreaves mide la respuesta a la estimulación por calor aplicado a la superficie plantar del animal. La fuente de calor se enfoca debajo de la superficie de la

planta y se registran la latencia de retirada de la pata y la intensidad infrarroja. Esta prueba permite la medición del umbral térmico nociceptivo agudo en animales de laboratorio (Hargreaves et al., 1988)

Al igual que en el test de von Frey, se estableció una línea basal previa a cualquier manipulación experimental. La habituación se realizó en cámaras de evaluación opacas individuales, similares a la usadas en el test de von Frey, y colocadas en un suelo de vidrio temperado a 30° C.

Para evitar la fobia al instrumento, durante días alternos de la fase precambio los animales fueron habituados al procedimiento durante 5 minutos después de las sesiones del CSNc. El día de la evaluación (día postcambio 1 o 2) y después de 15 minutos de habituación, se aplicó el estímulo en la planta de la pata trasera derecha o izquierda hasta que se produjo una respuesta de retirada. El reflejo de retirada interrumpe la luz reflejada desde la pata en una fotocélula y apaga automáticamente la luz y el temporizador. La intensidad de la luz se ajustó al comienzo de los experimentos a 85 mW/cm2 (equivalente a 42–43° C) con un radiómetro de flujo de calor, y esta intensidad no se cambió a lo largo de los experimentos. Cada rata se evaluó tres veces, y se usó un tiempo de latencia de corte de 20 s en cada medición. La temperatura se mantuvo constante durante todo el experimento.

La variable registrada fue la latencia de la respuesta de retirada (en segundos), como una medida indirecta del umbral de dolor por calor después de la retirada.

Además de las variables de registro (gramos en el caso de la prueba de von Frey y de segundos en el caso del test de Hargreaves) se tomaron anotaciones del comportamiento de la rata para determinar una posible diferencia en el comportamiento de la rata asociado con la experiencia de contraste.

2.4. Fármacos

La administración de fármacos se realizó en el Experimento 3. Los antagonistas del receptor sigma-1 utilizados fueron BD-1063 diclorhidrato y S1RA, mientras que los fármacos agonistas administrados fueron PRE-084 e igmesina.

Todos los fármacos se disolvieron en solución salina fisiológica (0.9% NaCl) y se alcalinizaron a un pH óptimo con NaOH. Todas las soluciones fueron preparadas

inmediatamente antes de comenzar los experimentos. Se administró 5 ml/kg del fármaco o su solvente por vía subcutánea (SC) en el área interescapular 30 minutos antes del procedimiento en el primer día de la fase postcambio. Tres días antes de la administración, los animales se habituaron a la manipulación que se lleva a cabo durante el día de la administración.

2.5. Análisis estadísticos

Para los análisis entre grupos, y en casos de violación de la normalidad y homocedasticidad de la muestra se llevaron a cabo pruebas no paramétricas para estudiar diferencias entre más de 2 grupos independientes (prueba Kruskall Wallis) y, asimismo, para estudiar diferencias entre 2 grupos independientes (prueba de U Mann-Whitney). Por otro lado, en los análisis intrasujetos se han realizado comparaciones mediante el Test de Friedmann.

Para los casos en los que se presenta una distribución normal y se ha cumplido el criterio de homocedasticidad, se han realizado pruebas paramétricas. Así, se ha realizado la prueba T-Student para medir diferencias entre 2 grupos independientes y ANOVA para más de 2 grupos. Por otro lado, se han llevado a cabo pruebas intrasujeto y contrastes polinómicos (análisis de tendencias) mediante la prueba de medidas repetidas.

En todos los casos, se consideraron diferencias estadísticamente significativas cuando el valor de $P < 0,05$.

3. Resultados y discusión

3.1. Experimento 1: Influencia de las experiencias previas en la respuesta emocional

Aquellos animales que no aprendieron la tarea fueron eliminados de los análisis. Así, se incluyeron 8 animales por grupo. El diseño fue de 3 (Grupo: Experimental, Incremento, Control) x 10 (sesiones). La variable dependiente registrada fue el tiempo en recorrer el laberinto durante todo el experimento.

Los resultados encontrados en este experimento han señalado varias implicaciones que se comentarán a continuación. En primer lugar, en la fase de precambio hay un

efecto de la cantidad de reforzador que obtiene el animal por realizar la tarea. De esta forma, los animales que han obtenido una mayor cantidad de refuerzo han sido más rápidos en recorrer el laberinto que aquellos que obtuvieron una menor cantidad de incentivo. Este efecto es algo conocido y ampliamente demostrado en este tipo de investigaciones (Flaherty, 1996). Más aún, el grupo de incremento ejemplifica a la perfección dicho efecto. Así, cuando la tasa de refuerzo en el grupo de incremento alcanza una cantidad de 8 *pellets*, las diferencias desaparecen entre el grupo experimental y el de incremento. Estos resultados también podrían mostrar la influencia que produce la forma de presentar el reforzador en los sujetos. Las diferentes formas de presentación crearían diferentes expectativas debido a las experiencias anteriores que ha tenido cada uno. Por lo referente al efecto de frustración asociado a la reducción en la cantidad de refuerzo obtenido tras la realización de la tarea, Flaherty et al. (1983) argumentaron que el hecho de que los roedores no hubieran desarrollado esa expectativa podría ser debido al paradigma experimental utilizado. En el CSNc el roedor se ve expuesto pasivamente al reforzador sin necesidad de realizar ninguna tarea. Por otro lado, el CSNi requiere que el roedor realice una tarea para poder obtener el reforzador. Volviendo nuevamente a los resultados obtenidos, las diferencias encontradas en el día postcambio 2 son sólo entre el grupo control y los grupos 12-2 y de incremento gradual, no diferenciándose entre sí estos últimos. Mientras que en el postcambio 3 solo se encontraron diferencias significativas entre el grupo control y el 12-2. Por tanto, debido a que el grupo 12-2 consumió una cantidad total mucho mayor de reforzador (360 *pellets*) en comparación al grupo de incremento gradual (252 *pellets*) durante la fase precambio, la frustración encontrada fue superior en el grupo experimental frente al de incremento. Los resultados expuestos apoyan que el animal pueda estar haciendo un promedio de la cantidad de reforzador administrado durante la fase precambio (360 vs 252 *pellets*). Asimismo, esto se ha hecho evidente en la devaluación del grupo experimental con un mayor reforzador (360 *pellets*) dado que ha mostrado una mayor frustración frente al grupo de incremento gradual (252 *pellets*) (McHose y Peters, 1975), siendo rechazada la hipótesis de la creación de una expectativa de adquirir cada día un reforzador mayor (Flaherty et al., 1998).

3.2. Experimento 2: Hipoalgesia inducida por la devaluación de recompensas

El Experimento 2 ha pretendido evaluar la percepción del dolor, mediante el test de von Frey o el test Hargreaves, en una tarea de CSNc.

Para los estudios de estimulación mecánica y térmica se llevó a cabo una fase precambio, la cual duró 6 días, en la que los grupos experimentales (el grupo experimental evaluado el día 1 o 32/17 y el evaluado el día 2 o 32/18) recibieron sacarosa al 32% frente a los controles que la recibieron al 4% (el grupo control evaluado el día 1 o 4/12 y el evaluado día 2 o 4/18). La fase postcambio duró solamente 3 días en los que todos los grupos recibieron sacarosa al 4%.

En primer lugar, se observó un contraste muy débil en comparación con los resultados típicos obtenidos en esta tarea por otros autores. Típicamente, Flaherty (1996) señalaba que el contraste dura entre 1 y 5 sesiones después de la primera sesión postcambio. Así, en este caso duró una sola sesión y la diferencia entre los grupos devaluados y controles fue relativamente pequeña en términos absolutos. La representación de los datos señala la existencia de una variabilidad individual asociada a la devaluación del reforzador (Ortega et al., 2014; Papini et al., 2015) responsable de la varianza sistemática primaria. A pesar de todo ello, el efecto débil del CSNc no redujo la hipoalgesia evaluada con la prueba de von Frey o el Hargreaves. Además, la hipoalgesia estuvo presente después de la sesión 18 en la evaluación de von Frey, en la cual no se había observado el contraste. Resultados similares se han encontrado en estudios de preferencia de etanol y CDP sobre el agua, inmediatamente después de una experiencia de devaluación de recompensa. Curiosamente, la preferencia por estas sustancias con efectos ansiolíticos persiste durante más tiempo que el efecto CSNc (Manzo et al., 2015). Estos resultados sugieren que incluso cuando los animales parecen recuperarse conductualmente de los efectos negativos de la devaluación de la recompensa en el comportamiento consumatorio, otras medidas (incluida la sensibilidad al dolor en el presente experimento) indican que todavía están emocionalmente afectados.

En segundo lugar, el problema de la selectividad del ensayo surgió por los resultados de un experimento similar al presente, en el cual se utilizó la placa caliente para evaluar la sensibilidad al dolor (Mustaca y Papini, 2005). En ese experimento, la

hipoalgesia apareció después de la segunda sesión postcambio, pero no se detectó después de la primera sesión postcambio. Un aspecto novedoso de nuestros resultados es el hallazgo de hipoalgesia inmediatamente después de la primera y segunda sesión de postcambio. Estos resultados sugieren que dependiendo del modelo utilizado se pueden observar diferencias en la sensibilidad al dolor (Yerasi y Manchikanti, 2019).

En tercer lugar, el estudio del curso de la hipoalgesia produjo un resultado inesperado: elevación de los umbrales de dolor 300 minutos después del final de la segunda sesión postcambio. Es posible que una segunda exposición a la devaluación reduzca un grado de excitación emocional, el cual no decayó por completo en los siguientes 300 minutos. De hecho, dado que el nivel de hipoalgesia después del segundo día postcambio, inmediatamente después de la devaluación frente a los 300 minutos es prácticamente idéntico, habría que suponer que no hubo descenso de activación emocional. Esto es difícil de corroborar, ya que, mediante una variedad de medidas, las consecuencias de la omisión de recompensas ya sean conductuales o fisiológicas, parecen decaer bruscamente en el tiempo, es decir, de segundos a minutos, dependiendo de la situación (Mitchell y Flaherty, 1998; Pecoraro et al., 2009).

Nuestros resultados demuestran la influencia que la frustración inducida por la devaluación de la recompensa parece tener sobre la sensibilidad al dolor físico. Aunque los mecanismos neurobiológicos subyacentes a dicha modulación son parcialmente conocidos (Papini et al., 2015), para una comprensión más completa de estos mecanismos sería necesario llevar a cabo estudios más específicos sobre este tema.

3.3. Experimento 3: Papel de los receptores sigma-1 en la devaluación de recompensas

El último experimento de esta tesis ha estudiado el efecto de la administración de diferentes fármacos agonistas y antagonistas de los receptores sigma-1 (Sig1R) en el procedimiento del CSNc. En todos los casos la administración ocurrió en un solo ensayo, 30 minutos antes de la primera sesión postcambio.

En primer lugar, los resultados obtenidos por los grupos tratados con suero fisiológico no afectaron al procedimiento de administración. De tal manera, que el grupo experimental mostró un incremento del consumo de sacarosa, el cual fue interrumpido por la devaluación de la recompensa alcanzando niveles inferiores de

consumo respecto al grupo control, es decir, mostrándose una frustración con una duración de 3 días. Estas diferencias entre los grupos durante la fase postcambio han sido difícilmente alcanzables en algunos grupos. Es decir, algunos grupos experimentales han presentado niveles similares al control en su consumo de sacarosa (concretamente, se ha visto en los grupos S1RA 16 mg/kg, BD1063 4 mg/kg, PRE-084 8 y 2 mg/kg e igmesina 4 mg/kg) que no llegaron a señalar diferencias significativas en dicho consumo entre los grupos (experimental *versus* control). Este hecho ha generado una considerable variabilidad de los resultados obtenidos en el primer día de la fase postcambio y, por lo tanto, en la interpretación de estos. Este efecto puede encontrarse claramente en el análisis estadístico de los grupos PRE-084 en las dosis de 8 y 4 mg/kg. Ambos grupos han mostrado una clara eliminación de la frustración en postcambio 1, pero que puede verse condicionado por la línea de base del grupo de control. Resultados similares se han encontrado con BD1063 16 mg/kg y 4 mg/kg. Por lo tanto, nuestros resultados después de la administración de los ligandos Sig1R podrían estar muy condicionados por estos aspectos experimentales del CSNc que son esenciales en este paradigma.

Teniendo en cuenta estos aspectos, se ha tratado de evaluar los efectos farmacológicos de los ligandos Sig1R utilizando un enfoque analítico diferente. De tal manera, que se ha utilizado el Ratio de Efectividad de la Droga o (RED) descrito por Flaherty (1996). La aplicación de la fórmula RED elimina esas diferencias (entre el día 10 precambio y el día 11 postcambio) y normaliza el consumo de grupos de control y experimentales (ver sección 2.2 Material y métodos). Por lo tanto, analizamos la eficacia de los ligandos Sig1R usando este enfoque de cálculo y los resultados obtenidos con estos fármacos en el paradigma CSNc serán discutidos considerando el RED.Flaherty y colaboradores (1980) encontraron una reducción de la frustración en el primer día postcambio después de la administración repetida de benzodiacepinas durante varios días antes del contraste (Flaherty et al. 1980). De acuerdo con la clasificación RED, el fármaco con la mejor efectividad fue el agonista PRE-084, dado que la dosis de 8 mg/kg indujo una eliminación total de la frustración actuando incluso en el primer día postcambio. En comparación con algunos resultados encontrados por Flaherty, PRE-084 8 mg/kg mostró una efectividad similar a la inducida por clordiazepóxido más etanol (Becker y Flaherty, 1983). Es importante resaltar que la administración de algunos ligandos Sig1R produjo una reducción de la frustración de la

primera sesión, incluso después de una sola administración 30 minutos antes del procedimiento.

Como se mencionó anteriormente, se ha encontrado que los agonistas Sig1R modulan estados como la depresión, la ansiedad, el aprendizaje y el deterioro de la memoria en diferentes modelos farmacológicos y patológicos en roedores (revisado por Cobos et al., 2008; Maurice y Goguadze, 2017). En particular, la igmesina y PRE-084 han demostrado reducir la depresión y mejorar el deterioro de la memoria con una sola dosis (Matsuno et al., 1996; Urani et al., 2001). Nuestros datos muestran que PRE-084 (en todas las dosis) ha sido el único fármaco que eliminó por completo la frustración, mientras que la igmesina también redujo la frustración a las dosis de 16 mg/kg y 1 mg/kg. La última dosis ha sido la única dosis que recuperó el consumo durante el postcambio a los niveles indicados en la fase precambio.

Por lo tanto, estos resultados sugieren un posible papel modulador de los agonistas Sig1R en los procesos de frustración.

Por otro lado, se ha demostrado una relación entre el dolor y la frustración (Mustaca y Papini, 2005; Ortega et al., 2011). En concreto, se ha encontrado una menor sensibilidad al dolor o hipoalgesia después de una exposición al paradigma CSNc (revisado por Papini et al., 2015). De hecho, los resultados de nuestro experimento 2 respaldan estos hallazgos previos relacionados con la hipoalgesia inducida por los procesos de frustración. Además, existe una convergencia anatómica y fisiológica entre el dolor, las emociones negativas y la motivación, conocida como hipótesis de control adaptativo (Shackman et al., 2011). Así, numerosos estudios han comprobado el papel del antagonismo Sig1R en la reducción o eliminación de diferentes tipos de dolor (Zamanillo et al., 2013; Merlos et al., 2017).

La evaluación de los antagonistas Sig1R (BD1063 y S1RA) en el CSNc ha generado resultados contradictorios. Mientras que la administración del antagonista Sig1R BD1063 a dosis de 16 mg/kg y 1 mg/kg indujo una reducción de la frustración, lo que respalda la hipótesis del control adaptativo (Shackman et al., 2011), la administración del S1RA no modificó la respuesta de frustración con ninguna de las dosis probadas. Esta aparente discrepancia puede estar relacionada con la falta de diferencias previas a la devaluación entre los grupos experimentales y control, como discutimos anteriormente. Además, aunque la administración de PRE-084 ha

demostrado potenciar las respuestas nociceptivas en varios modelos de dolor (Roh et al., 2008; Entrena et al., 2016), en nuestro estudio, no aumentó la frustración. Además, la incapacidad de S1RA para reducir o eliminar la frustración en el primer día postcambio puede indicar que no hay dolor físico el primer día postcambio. Por lo tanto, el dolor psicológico (relacionado con los procesos de frustración) descrito por Papini y colaboradores (2015) en términos de "un estado aversivo inducido por la omisión o devaluación de la recompensa real o anticipada en un contexto no social" sugiere otro tipo de efecto negativo que no implicaría al dolor físico (Papini et al., 2015). Estos antecedentes también respaldan que los efectos del PRE-084 y la igmesina sobre la frustración pueden estar relacionados con sus acciones ansiolíticas y no con sus efectos relacionados con el dolor.

Nuestros resultados obtenidos tras la administración de ligandos Sig1R sugieren que la frustración, inducida por CSNc, no implica un dolor físico sino la posible modulación de respuestas relacionadas con la ansiedad, la memoria y el aprendizaje. La gran eficacia de las benzodiacepinas para reducir la frustración en el segundo día postcambio apoyaría la aparición de respuestas de ansiedad causadas mediante la exposición repetida a procesos de frustración. Sin embargo, se sugiere la existencia de otros procesos que requieren memoria y aprendizaje en el primer día postcambio.

En resumen, encontramos que los agonistas de Sig1R parecen mitigar la frustración en el paradigma de CSNc, mientras que las acciones de los antagonistas de Sig1R son contradictorias. Sin embargo, debido a la gran variabilidad observada durante el procedimiento de CSNc, se necesitan más estudios para respaldar nuestros datos, por ejemplo, evaluando algún compuesto Sig1R adicional, o bien, estudiar animales *knockout* de Sig1R en este modelo.

4. Conclusiones

Los resultados obtenidos arrojan una serie de conclusiones en función de las hipótesis realizadas al inicio de la Tesis. En primer lugar, se han desarrollado las conclusiones específicas obtenidas a partir de los resultados y, posteriormente, se presentan las conclusiones generales de la presente Tesis Doctoral.

4.1. Conclusiones específicas

1. La manipulación experimental del reforzador (número de *pellets*) durante la fase precambio en el paradigma CSNi, aumentando gradualmente el reforzador, produce un efecto de contraste débil en comparación con el grupo experimental, en el cual se produce una frustración de 3 días después de la devaluación del reforzador.
2. La frustración inducida por la devaluación de la recompensa en el paradigma CSNc induce una disminución de la sensibilidad al dolor (es decir, hipoalgesia) provocada por un estímulo mecánico (prueba de von Frey) y un estímulo térmico (prueba de Hargreaves).
3. La frustración provoca una hipoalgesia que se mantiene inmediatamente después de la devaluación y a las 5 horas, solo en el segundo día de contraste, independientemente del estímulo aplicado para la evaluación (mecánico o térmico).
4. La administración sistémica de ligandos Sig1R modula diferencialmente la frustración inducida por el CSNc. Las acciones diferenciales observadas parecen, en algunos casos, estar mediadas por la falta de diferencias entre el control y los grupos experimentales en las sesiones previas y posteriores a la devaluación en el CSNc.
5. La administración subcutánea de los agonistas selectivos de Sig1R (PRE-084 e igmesina) reduce o elimina la frustración tanto en el primer como en el segundo día de la fase postcambio.
6. La administración subcutánea de los antagonistas selectivos de Sig1R produce resultados controvertidos, debido a que BD1063 parece reducir la frustración, mientras que S1RA no modifica la respuesta de frustración en ninguna de las dosis probadas.

4.2. Conclusión general

La frustración es un fenómeno que puede mitigarse si previamente se han tenido experiencias con bajos niveles de refuerzo.

El CSNc ha demostrado ser un modelo muy complejo. Mientras que del primer al segundo día la respuesta conductual se va atenuando, la respuesta registrada mediante la

evaluación fisiológica tiende a presentarte a lo largo del tiempo. Dicha respuesta fisiológica se mantiene en el segundo día tras 300 min después de la devaluación del reforzador.

La frustración implica procesos como la memoria, el aprendizaje y la ansiedad. La reducción de la frustración en postcambio 1 y 2 a través de una sola administración de fármacos agonistas Sig1R parece apoyar esta hipótesis, mientras que los fármacos antagonistas Sig1R han mostrado resultados controvertidos.

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Abbreviations

BD-1047	N-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(dimethylamino)ethylamine dihydrobromide
BZD	Benzodiacepines
CDP	Chlordiazepoxide
CNS	Central Nervous System
CSN	Contraste Sucesivo Negativo
CSNc	Contraste Sucesivo Negativo consumatorio
cSNC	Consummatory Sucesive Negative Contrast
CSNi	Contraste Sucesivo Negativo instrumental
DER	Drug Effectiveness Ratio
ER	Endoplasmic Reticulum
i.p.	Intraperitoneal
Igmesine	[(+)-cinnamyl-1-phenyl-1-N-methyl-N-cyclopropylene]
iSNC	Instrumental Sucesive Negative Contrast
ITI	intertrial interval
JO-1784	[(+)-cinnamyl-1-phenyl-1-N-methyl-N-cyclopropylene]
K+	Potassium
Na+	Sodium
PRE-084	2-[4-morpholinethyl]1- phenylcyclohexanecarboxylate hydrochloride
s.c.	Subcutaneous
s1	Sigma 1
S1RA	4-[2-[[5-methyl-1-(2-naphtalenyl)1H-pyraol-3-yl]oxy]ethyl]
s2	Sigma 2
Sig1R	Sigma 1 Receptor
Sig2R	Sigma 2 Receptor
SNC	Sucesive Negative Contrast

List of publications

PUBLISHED ARTICLES

1. Articles directly relacted to this doctoral thesis

Jiménez-García, A. M., Ruiz-Leyva, L., Vázquez-Ágredos, A., Torres, C., Papini, M. R., Cendán, C. M. and Morón, I. (2019). Consummatory Successive Negative Contrast in Rats. *Bio-protocol 9*(7): e3201. DOI: 10.21769/BioProtoc.3201.

Jiménez-García, A. M., Ruíz-Leyva, L., Cendán, C. M., Torres, C., Papini, M. R., & Morón, I. (2016). Hypoalgesia induced by reward devaluation in rats. *PloS one, 11*(10). https://doi.org/10.1371/journal.pone.0164331

2. Other articles

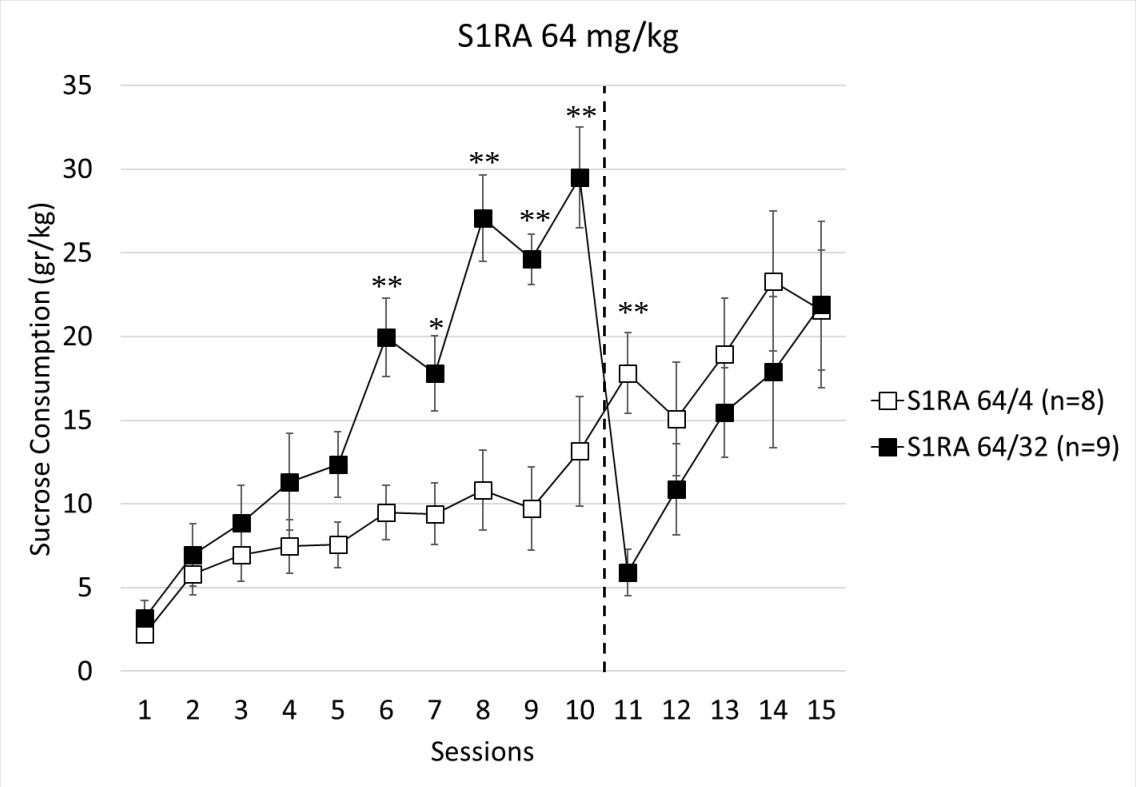
Fernández, M. S., Bellia, F., Ferreyra, A., Chiner, F., Jiménez-García, A. M., D'Addario, C., & Pautassi, R. M. (2020). Short-term selection for high and low ethanol intake during adolescence exerts lingering effects in stress-induced ethanol drinking and yields an anxiety-prone phenotype. *Behavioural brain research*, *380*, 112445.

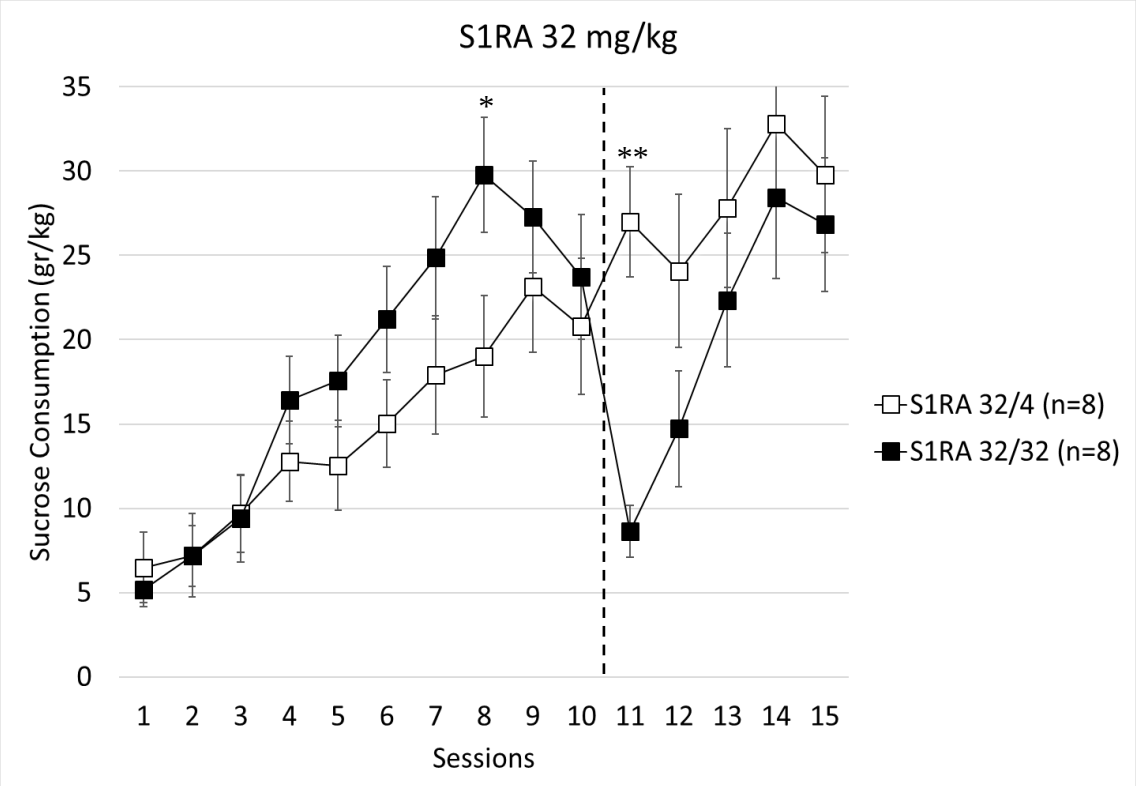
Wille-Bille, A., Bellia, F., Jiménez-García, A. M., Miranda-Morales, R. S., D'Addario, C., & Pautassi, R. M. (2020). Early exposure to environmental enrichment modulates the effects of prenatal ethanol exposure upon opioid gene expression and adolescent ethanol intake. *Neuropharmacology*, *165*, 107917.

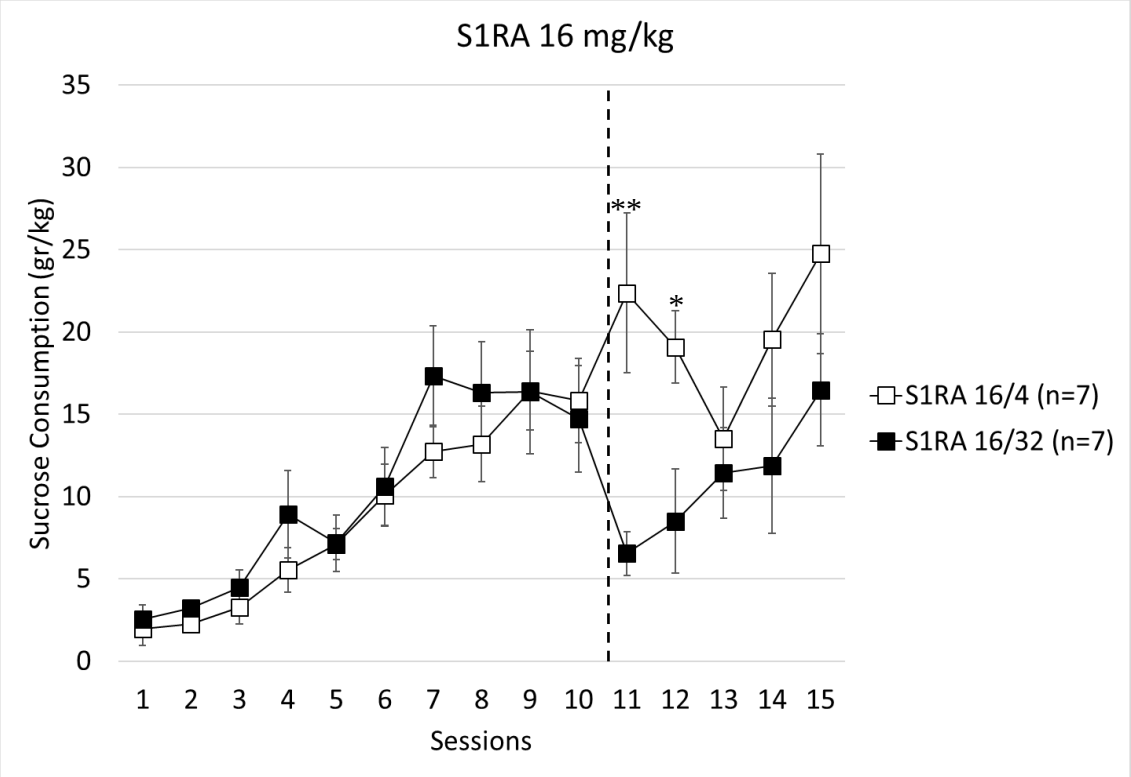
Correa, A., Alguacil, S., Ciria, L. F., Jiménez-García, A.M, & Ruz, M. (2020). Circadian rhythms and decision-making: a review and new evidence from electroencephalography. *Chronobiology international*, 1-22.

Annexes

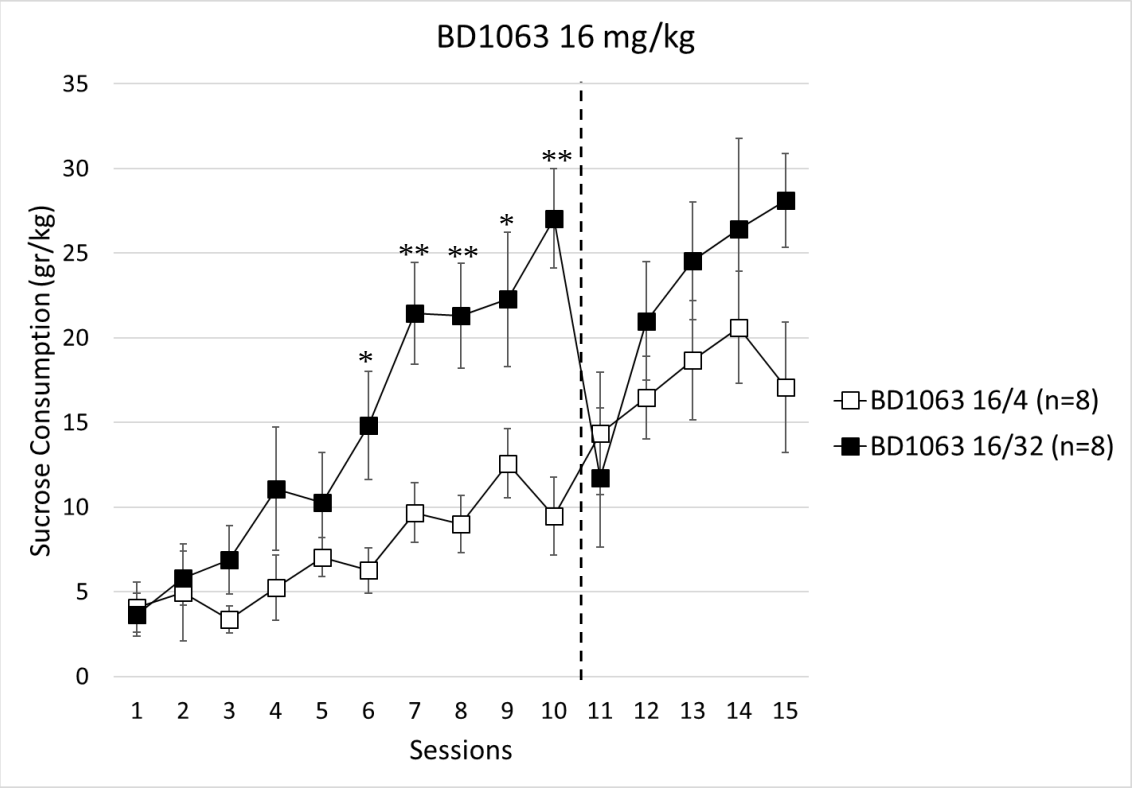
Annex 1. S1RA administration in Consummatory Successive Negative Contrast.

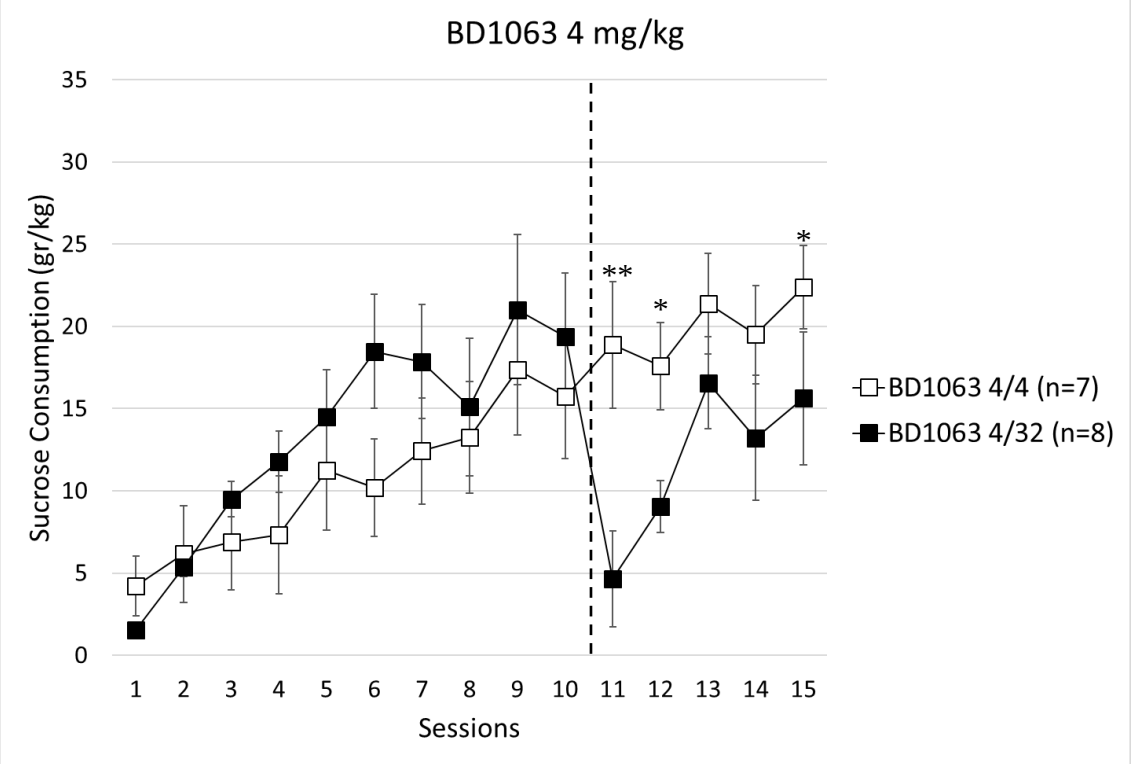


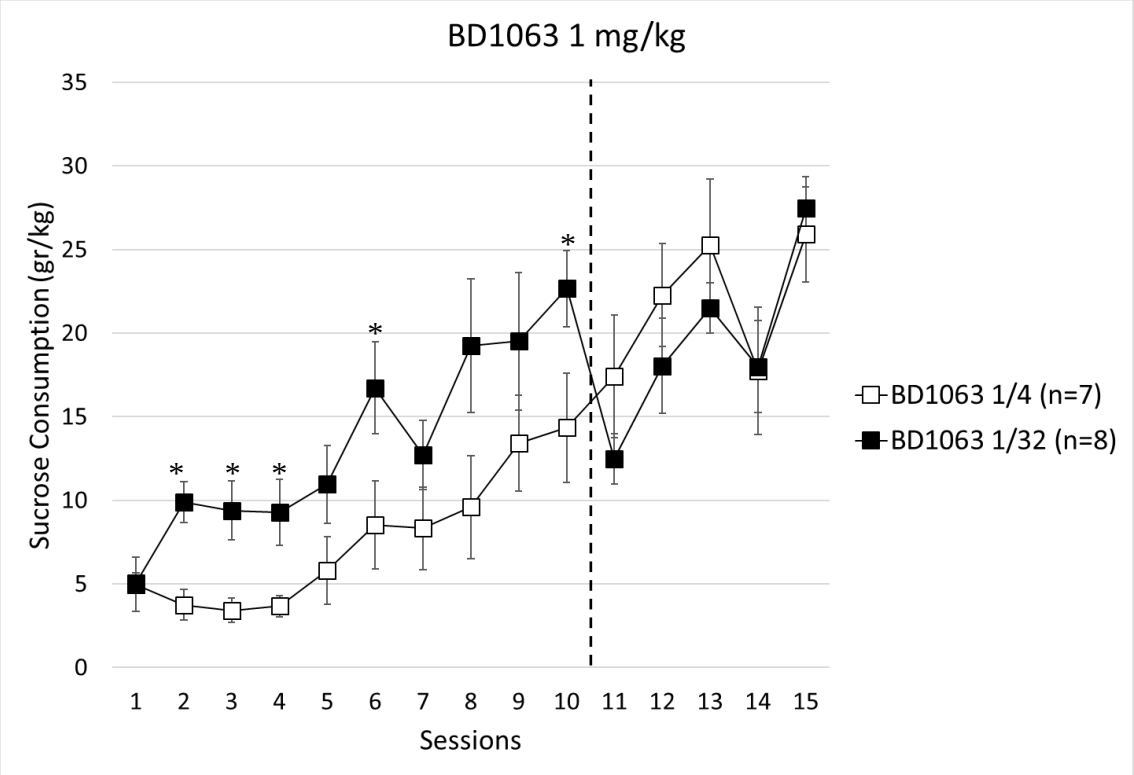



S1RA 16 mg/kg
Sucrose Consumption (gr/kg)
35
30
25
20
15
10
5
0
**
*
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Sessions
S1RA 16/4 (n=7)
S1RA 16/32 (n=7)

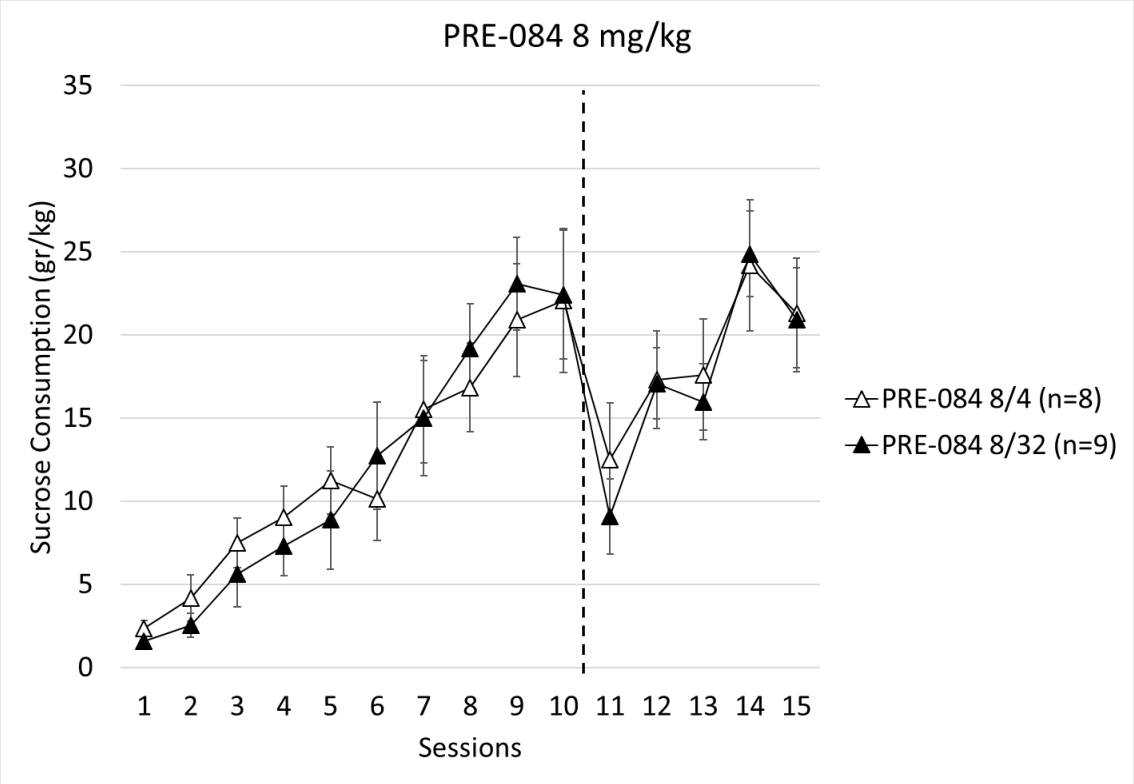
Annex 2. BD1063 administration in Consummatory Successive Negative Contrast.

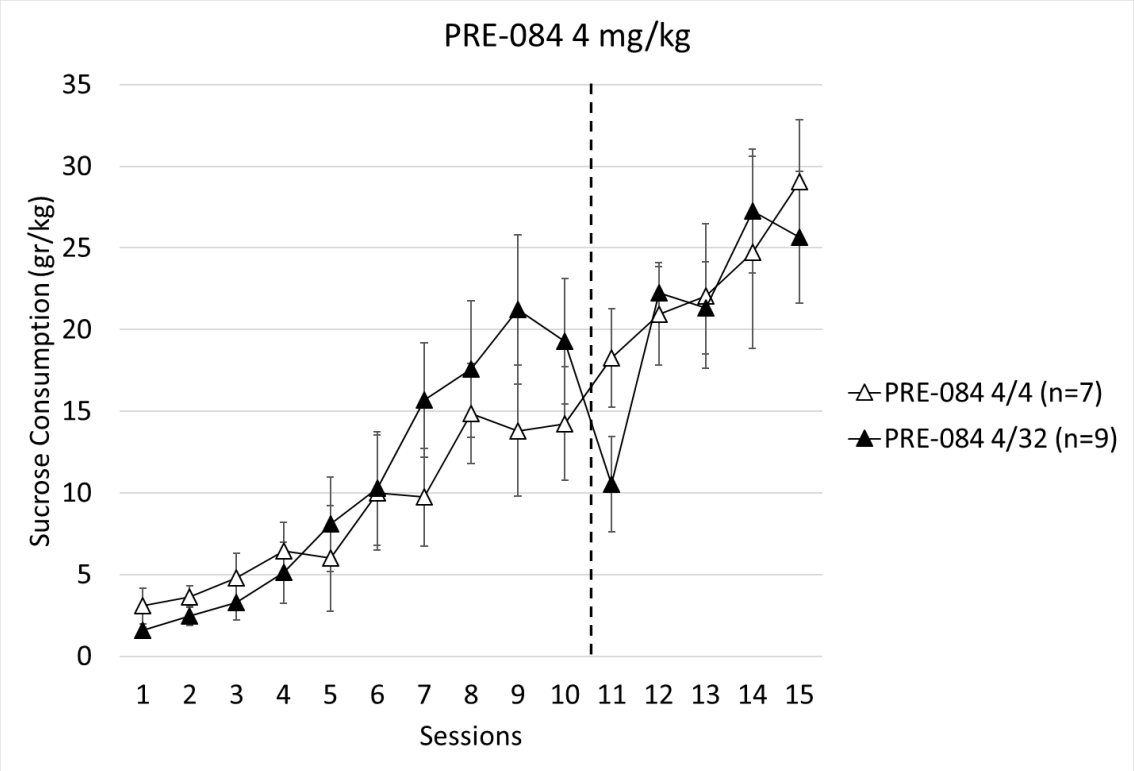


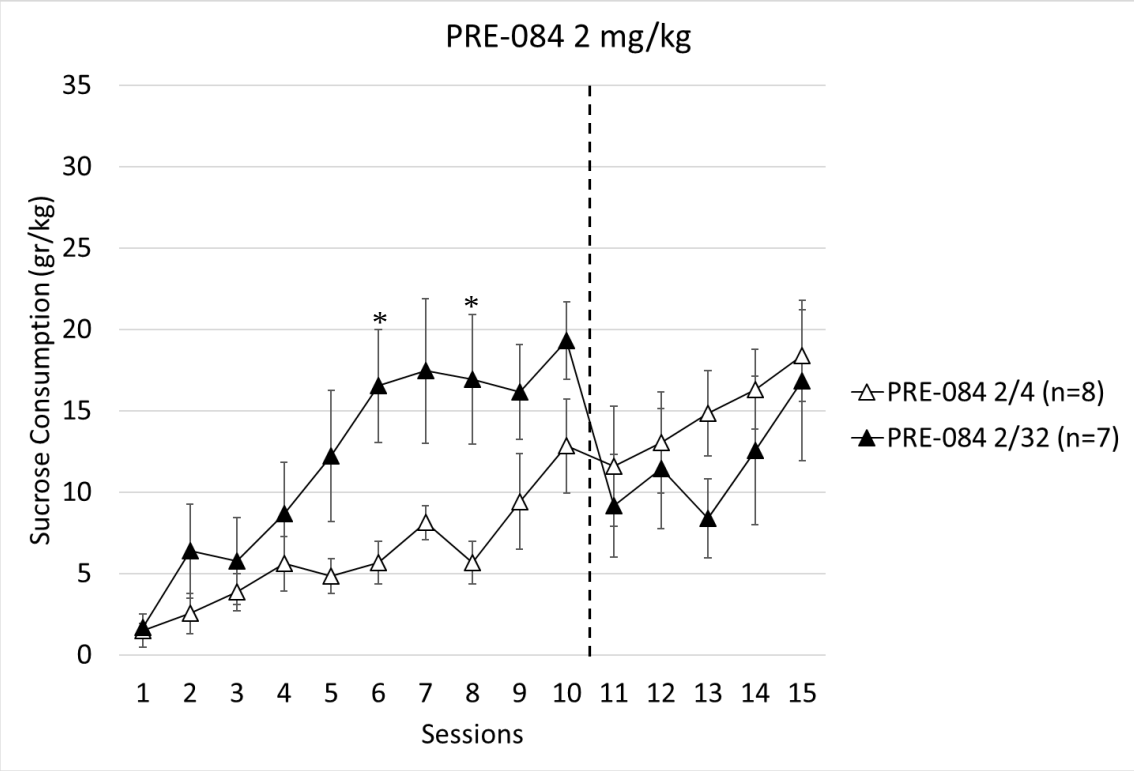



BD1063 1 mg/kg
Sucrose Consumption (gr/kg)
35
30
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Sessions
*
*
*
*
*
BD1063 1/4 (n=7)
BD1063 1/32 (n=8)

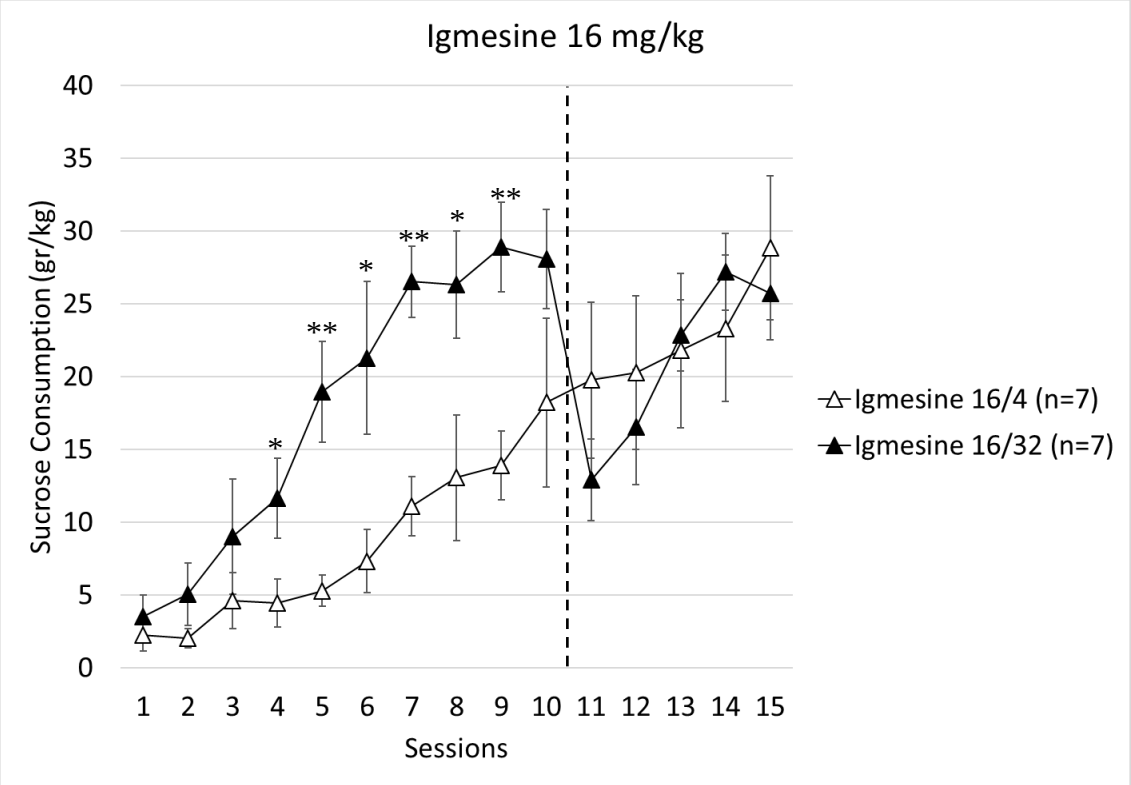
Annex 3. PRE-084 administration in Consummatory Successive Negative Contrast.

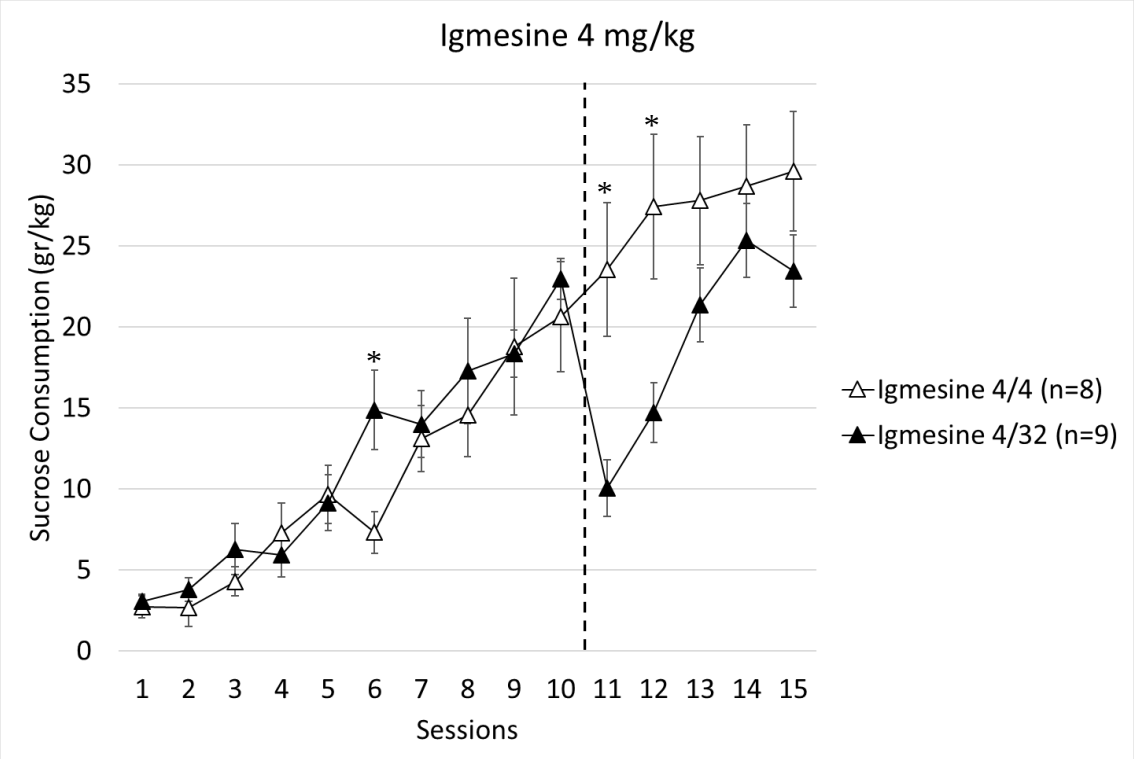


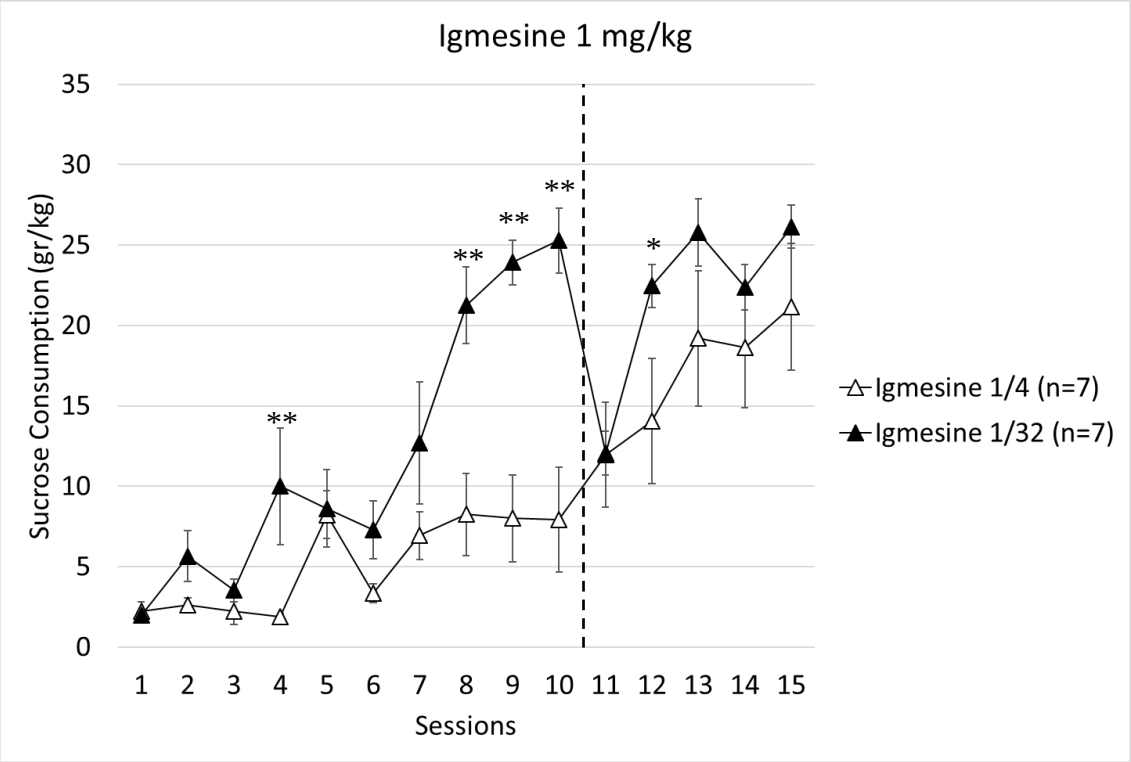



PRE-084 2 mg/kg
Sucrose Consumption (gr/kg)
35
30
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Sessions
*
*
PRE-084 2/4 (n=8)
PRE-084 2/32 (n=7)

Annex 4. Igmesine administration in Consummatory Successive Negative Contrast.






Igmesine 1 mg/kg
Sucrose Consumption (gr/kg)
35
30
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Sessions
**
**
**
**
*
Igmesine 1/4 (n=7)
Igmesine 1/32 (n=7)