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Effects of Selective Neurotensin Receptor Antagonists on Responding for Brain Stimulation Reward

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ABSTRACT

Effects of Selective Neurotensin Receptor Antagonists on Responding for Brain Stimulation Reward

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Electrical stimulation of the medial mesencephalic central gray produces a strong rewarding effect, and laboratory animals can easily be trained to produce an operant response to obtain such a stimulation. Previous studies have shown that the rewarding effect of mesencephalic electrical stimulation is potentiated by intracerebroventricular and by ventral mesencephalic microinjections of neurotensin (NT); these findings suggest that this neuropeptide may be an important component of the mesencephalic reward-relevant pathway. The present work was aimed at testing this hypothesis by studying the effects of systemic administration of two selective NT antagonists, SR-48692 and SR-142948a, on responding for medial mesencephalic electrical stimulation. The curve-shift method adapted to operant responding for brain stimulation reward was used to assess reward and performance changes following intra-peritoneal injections of four doses (40, 80, 160, and 3000 μg/kg) of SR-48692, three doses (40, 160, 640 μg/kg) of SR-142948a, and the vehicle. Results show that SR-48692 and SR-142948a did not alter rewarding effectiveness of the stimulation nor the maximal rates of operant responding at any of the doses tested. These results raised the following hypotheses: 1) NT

is not a component of the reward-relevant pathway activated by mesencephalic electrical stimulation; 2) reward-induced by mesencephalic electrical stimulation is not under tonic control of endogenous NT, and 3) NT is a component of the reward-relevant pathway but it acts through subtype(s) of NT receptor(s) that is/are not antagonized by SR-48692 and SR-142948a.

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Dedication

This thesis is dedicated to my grandmother Germaine.

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According to Skinner, objects or events that change the frequencies of an operant response are said to have reinforcing properties. Stimuli that initiate approach have a positive, or rewarding, effect whereas stimuli that initiate avoidance have a negative, or aversive effect. Both positive and negative reinforcers increase the probability of occurrence of a behavioural response. This contrasts with the concept of punishment, which refers to the presentation of an aversive stimulus following a behavioural response; hence punishment decreases the probability of occurrence of a behavioural response. Reinforcers, either negative or positive, play a crucial role in survival of the organism and of the species (Liebman, 1989, p.1). For example, a positive reinforcing effect occurs when an animal comes to associate food with a particular behavioral pattern (i.e. digging) displayed in a particular environment; this association increases the probability that the animal will return to this environment and display a similar behavioural pattern when she or he is hungry. A negative reinforcing effect occurs, however, if the animal is attacked by a predator when searching for food in the same environment; the animal may tend, in the future, to avoid this environment even though she or he had found food there in the past. Occurrence of both events, finding of food and being attacked by a predator will likely result in an approach-avoidance situation. An animal's behaviour is then determined, at least in part, by the strength of each of the motivational components.

The term positive reinforcement and reward are often used interchangeably although they do not have exactly the same significance. According to White (1989):

> "reward refers to the fact that certain environmental stimuli have the property of eliciting an approach response" whereas reinforcement refers to "the tendency of certain stimuli to strengthen a learned stimulus-response association" (p. 181).

In the present work, the term reward will be used to designate a stimulus that elicits an approach and increases the probability of occurrence of an operant response.

Reward tends to shape human and animal behaviour in everyday life. Hence, it plays a key role in how the organism adapts to its environment. In view of the importance of reward for survival of the organism, and of the species, a substantial amount of research has been dedicated to the understanding of its neural bases. Revealing the neural bases of reward can help develop better therapeutic treatments for disorders involving dysfunction(s) of the reward system such as depression, compulsion and substance abuses; it can also shed

light on the neural mechanism(s) responsible for normal appetitive behaviours such as food and water intake.

One of the models that has been developed to study the neural bases of reward is the operant responding for electrical brain stimulation, known as intracranial self-stimulation (ICSS) or brain stimulation reward (BSR). This model has emerged from the evidence provided by Olds and Milner (1954), 45 years ago, that electrical stimulation of some parts of the brain can act as a positive reinforcement. In the process of studying the effects of electrical brain stimulation on the behavioural response of a rat, Olds and Milner inferred that the stimulation likely had a rewarding effect because the rat had the tendency to remain in the area of the cage associated with the delivery of the stimulation. Their inference was confirmed when they successfully trained the rat to self-administer the electrical stimulation in an operant box. The discovery of BSR has had important impacts in the field. First, it provided evidence that neural elements in the brain can initiate approach behaviour in a similar way to behaviour elicited by natural incentive stimuli (e.g. food and water) when they are directly activated. Second, BSR provided physiological psychologists with a more direct access to the reward-relevant neural elements in the brain, allowing them to 1) characterise its physiology, anatomy and pharmacology and 2) test the hypothesis that natural stimuli produce their rewarding effect by activating reward-relevant neurons, via sensory afferents. In the following section, the different methods used to assess the rewarding effectiveness of the stimulation will be described and discussed.

Subsequently, studies that were aimed at identifying the neural bases of BSR will be reviewed.

Assessment of the rewarding effectiveness of the stimulation.

In the early BSR studies, measurements of rates of responding at fixed stimulation parameters recorded during a discrete time interval was used to infer the rewarding effectiveness of the stimulation. For instance, high effectiveness of the stimulation was inferred by a high rate of responding and low effectiveness of the stimulation was inferred from a low rate of responding. A main advantage of this method was its easy and inexpensive implementation. Later, however, it was demonstrated that the rate of responding does not reliably reflect the rewarding effectiveness of the stimulation. Hence, Hodos and Valenstein in 1962 showed that when the animal was given the choice between a stimulation into the septal area or into the hypothalamus, it preferred the stimulation into the septal area; this in spite of the fact that its rate of self-administration into the septal area was much lower than its rate of self-administration into the hypothalamus. The lower response rates for septal stimulation were explained by the presence of post-stimulation behavioural inhibition, a phenomenon not produced by hypothalamic stimulation. Hodos and Valenstein concluded that a time dependent method was not appropriate for comparing the rewarding effectiveness of the stimulation at two different brain sites.

A new method, called the curve-shift method, was then developed to better assess the rewarding effectiveness of the stimulation and to minimize the distortion of the assessment of the rewarding effectiveness of the stimulation by performance variables. The adaptation of the curve-shift method for BSR was first proposed by Edmond and Gallistel in 1974 who used the runway paradigm. In this paradigm, the animal is placed in a closed compartment at one end of an alley, and is given a few electrical stimulation as a priming stimulation. Following the priming stimulations, the starting compartment is opened and the animal is allowed to run down the alley towards a lever. Depression of this lever delivers a short train of brain stimulation that acts as a reward. The dependent variable is the speed at which the rat runs down the alley to reach the lever. Edmonds and Gallistel showed that the running speed is a function of the strength of the rewarding stimulation, and the function takes a sigmoid shape. This means that 1) the rewarding stimulation must have a minimal value to initiate running, 2) over a certain range of stimulation, the running speed increases monotonically with the strength of the stimulation and 3) the running speed reached a plateau at high stimulation strength. As an index of the rewarding effectiveness of the stimulation, Edmonds and Gallistel had proposed the stimulation frequency required to produce a running speed equal to 50% of the maximal speed, the M50 index. An important observation was that independent variables that alter performance were found to have small to moderate (0.1-0.3 log unit) effect on the M50 index. That is, increasing the slope of the alley, or adding hurdles in the middle, had much larger suppression effect on maximal running speed than on

the M50 index. These findings led Edmonds and Gallistel to infer that changes in M50 index in the presence of minimal changes in maximal running speed reflect changes in rewarding effectiveness of the stimulation, while small changes in M50 index in the presence of a large suppression in maximal running speed reflect changes in performance. Edmonds and Gallistel (1977) used the curve-shift method with the runway paradigm described above to test the effects of alpha-methyl-para-tyrosine, an inhibitor of the synthesis of the catecholamines. They found that this drug suppresses the rewarding effectiveness of the stimulation as reflected by an increase in M50 index; this marked the first use of the curve-shift to investigate pharmacology of BSR.

Yeomans (1975) was the first to adapt the curve-shift method to the operant box. In the operant box, the animal is given the opportunity to self-administer the electrical stimulation by pressing on a lever protruding from one wall of the box; this paradigm was first used by Olds and Milner to demonstrate the rewarding property of electrical brain stimulation as described above. The dependent variable is the number of lever presses produced during a single discrete trial. As previously shown by Edmonds and Gallistel (1974) with the runway paradigm, the strength of the operant response, the number of lever presses, is a function of the strength of the stimulation, a function that also takes the shape of a sigmoïd. Similarly to Edmunds and Gallistel (1974), Miliaressis et al. (1986) tested the sensitivity of the M50 index and of the maximal rate of responding to changes in performance variables and obtained very similar

results. For instance, injection of a muscle relaxant, increase in lever weight or imposition of a fixed interval schedule, moderately altered the M50 index (0.1-0.2 log unit), but did, as expected, produced large suppression of maximal rate.

In addition to providing a reliable measure of the rewarding effectiveness of the stimulation (the M50 index), the curve-shift method allows the experimenter to assess changes in performance produced by physiological or pharmacological treatments aimed at characterising the reward-relevant neural systems: Such a dissociation between reward and performance could not be achieved with the simple rate measure as used in most of the studies performed during the decades that followed Olds and Milner's discovery.

Location of reward-relevant sites in the central nervous system.

Following Olds and Milner's discovery, a significant amount of research has been dedicated to localizing within the central nervous system (CNS), sites that when electrically stimulated produces a rewarding effect. In the last forty years most of the major areas of the brain have been explored; a summary of the findings are presented below.

In the telencephalon, BSR has been induced in most of the cortical and subcortical areas that belong to the limbic system those areas are: the prepyriform cortex, the entorhinal cortex, the sulcal prefrontal cortex and the striatum (Prado-Alcala and al. 1984), the medial prefrontal cortex (Robertson,

1982), the cingulate cortex (Mekarski 1988), the hippocampus (Olds and al. 1960), the septum (Newman, 1961), the amygdala (Wurtz and Olds, 1963), the nucleus accumbens (Olds and al. 1960), the olfactory bulb (Routtenberg, 1971), the caudate and the putamen (Wurtz and Olds,1963) and the ventral pallidum (Panagis, 1995).

More recently, detailed mapping of positive BSR sites has been performed using moveable stimulation electrodes and psychophysical measures; this methodology consists of determining at successive dorso-ventral sites, the stimulation intensity, or the stimulation frequency, required to induce a fixed rate of responding for BSR. It provides two major advantages over the traditional method used in the initial mapping studies. First, it allows one to test, and to compare, several sites within the same subject, hence to increase histological resolution. Second, it decreases the occurrence of false negatives, through the use of fixed stimulation parameters.

Three studies have made used of moveable stimulation electrodes and psychophysical measures to map positive BSR sites in the telencephalon. Prado-Alcala et al. (1984) mapped the septal nucleus, and the medial frontal cortex. They found that 114 (90%) of the 127 sites tested in the lateral septal area sustained BSR. The sites with lower thresholds were found in the anterior-dorsal part of the nucleus. In the medial frontal cortex, 53 (67%) of the 69 sites tested sustained BSR while in the sulcal cortex, 12 (60%) of the 20 sites tested

sustained BSR. In the entorhinal cortex, (81%) of the 36 sites tested sustained BSR. In the parietal cortex out the seven sites tested, none of the sites sustained BSR. When these cortical regions were compared, the lowest thresholds were found in the medial frontal cortex.

In another study, Prado Alcala et al. (1984) found that BSR could be produced throughout the striatum and its adjacent areas. In the caudate-putamen 135 (48%) of the 280 sites tested supported BSR; the lowest thresholds were found in the anterior, ventral and medial areas. In the ventral striatum 29 (97%) of the 30 sites tested supported BSR and the lowest thresholds were found ventro-medially. In the olfactory tubercles 14 (78%) of the 18 sites investigated sustained BSR; the lowest thresholds were found in the anterior, ventral and medial areas. In the amygdala, 22 (92%) of the 24 sites investigated sustained BSR. The medial nucleus was more sensitive than the lateral, and according to Kane et al. (1991) the medial nucleus contains a homogenous distribution of the reward-relevant neurons.

More recently, Panagis et al. (1995) mapped reward-relevant sites within the ventral pallidum and its surrounding areas. The surrounding areas were: the globus pallidus, the nucleus horizontal, the limb diagonal band, the magnocellular preoptic nucleus, the anterior amygdaloid area, the caudate-putamen, the substantia innominata and the olfactory tubercle. They found that 104 (79%) of the 131 of the sites tested supported BSR. The lowest thresholds were found in

the medial region of the ventral pallidum, a region that receives afferents from the ventral striatum and sends efferents to the ventral tegmental area. This is noteworthy because the ventral striatum and the ventral tegmental area are two regions known to sustain BSR.

In the ventral diencephalon, BSR has been induced by stimulation of the lateral preoptic area (German and Bowden, 1974), the anterior hypothalamus, the lateral hypothalamus, and near and around its adjacent nuclei and pathways such as the fornix, the zona inerta, the medial tip of the internal capsule (Gratton and Wise, 1983), the posterior hypothalamus (German and Bowden, 1974) and the subfornical organ (Robertson, et al. 1977). In fact, BSR can be induced by stimulation of a major pathway that links telencephalic to mesencephalic nuclei, the medial forebrain bundle (MFB). In most of the BSR studies the MFB has been the standard stimulation site. Within the dorsal diencephalon, BSR can be induced by stimulation of some midline thalamic nuclei, the dorsomedial, the paraventricular, the reuniens, the ventromedial (Clavier, 1982), and the habenular nuclei (Vachon and Miliaressis, 1992), all along the stria medullaris, a dorsal pathway that links the habenular complex to some ventral telencephalic nuclei (Blander and Wise, 1989).

In the anterior diencephalon, Blander and Wise (1989) revealed the existence of a cluster of positive sites within and around the stria medullaris and the anterior hypothalamus; those areas that produced the lowest thresholds were

in the lateral part of the anterior hypothalamus. Within the lateral hypothalamus, Gratton and Wise (1983) found lowest thresholds between the zona inerta and the base of the brain, and between the fornix and the internal capsule. They also reported positive sites in the dorso-medial and ventromedial areas of the hypothalamus but with much higher thresholds.

In the dorsal diencephalon, Vachon and Miliaressis (1992) found positive BSR sites in the mediodorsal, paratenial, interanteromedial, centromedial, reuniens and the rhomboid nucleus of the thalamus as well as in the stria medullaris. The lowest thresholds were found at the junction between the stria medullaris and the lateral habenula nucleus, in the most rostral portion of the dorsal diencephalon; these findings agree with those reported by Blander and Wise (1989).

In the mesencephalon, several studies have confirmed the existence of positive BSR sites within the most ventral areas, namely the substantia nigra (Crow, 1972), and the ventral tegmental area (Ball, 1972). More posteriorly, positive BSR sites are found more dorsally within the periaqueductal grey (Cooper and Taylor, 1967), the mesencephalic nucleus of the trigeminal nerve (Van Der Kooy and Phillips, 1977), the dorsal raphe (Simon et al, 1976) and the median raphe (Simon and al. 1973).

Wise (1981) attempted more specifically to map the location of BSR positive sites just above and within the pars compacta and reticulata of the substantia nigra. He reported that 59 (17%) of the 351 sites investigated supported BSR; the lowest threshold were found anteriorly within the pars compacta of the substantia nigra. No positive sites were found in the zona recticulata and in the medial lemniscus.

In the caudal mesencephalon, Corbett and Wise (1979) reported forty-two (53%) of the 79 sites in the dorsal raphe or its adjacent areas supported BSR. The lowest threshold were found in the dorsal raphe, and in areas rostral to the dorsal raphe. Positive BSR sites were also found in the superior cerebellar peduncle and in the motor nuclei of the trigeminal nerve. Interestingly, no positive sites were found in the locus coeruleus and in the lateral areas of the superior cerebellar peduncle, findings that contrast with earlier studies (see German and Bowden, 1974).

In the medial mesencephalon, Rompré and Miliaressis (1985) found a large and continuous band of positive sites on the midline, between the anterior-posterior level of the dorsal raphe and the ventral tegmental area. At the level of the dorsal raphe nucleus, the lowest thresholds were located within the decussation of the superior cerebellar peduncle, and more rostrally within the ventral tegmental area.

In the metencephalon and myelencephalon, positive BSR sites have been found in the trigeminal motor nucleus (Van Der Kooy, 1979), the most caudal part of the central grey (Boye and Rompré, 1989), the parabrachial area (Ferssiwi and al.,1987), the solitary tract (Carter and Philips, 1975), within, and around, the deep cerebellar nuclei (Ball and al. 1974; Corbett and al. 1982), in the superior cerebellar peduncle (Routtenberg and Malsbury, 1969), and around the locus coeruleus (Crow and al. 1972). According to Rompré and Boye (1989), the caudal and lateral areas of the pontine tegmentum are mostly devoid of reward-relevant neurons as only 9 (5%) of the 181 sites investigated sustained BSR. The few positive sites were found in the rostro-lateral pontine regions, near and within the superior cerebellar peduncle. Self-stimulation could not be induced by stimulation of locus coeruleus, a finding consistent with Corbett and Wise's report (1979).

Areas of the cerebellum were investigated by Corbett et al. (1982) who found 10 positive sites (36%) out of the 28 sites investigated. The positive sites were found between the ventral fastigial nucleus anteriorly and the cerebellar medulla on the lateral borders of the vermian lobules I (lingula) and II (lobus centralis).

Physiological characteristics of the reward-relevant neuron

In an attempt to identify the directly stimulated reward-relevant neurons within the MFB, some researchers have adapted techniques currently used to

measure axonal physiological properties such as the refractory period and the conduction velocity to the study of BSR. Results of these studies have revealed that MFB reward-relevant neurons travel between the lateral hypothalamus and ventral tegmental area. And that they have refractory periods and conduction velocities that range from 0.5 to 1.2 msec and 2 to 8 m/sec respectively (see Gallistel et al., 1981). Taking advantage of another technique used in standard electrophysiological experiments, Bielajew and Shizgal (1986) also obtained indirect evidence that at least one group of MFB reward-relevant neurons has axons that travel in a rostro-caudal direction. Taken together these results suggest that the MFB reward-relevant neurons are small, myelinated neurons that likely originate from rostral basal forebrain regions and project towards the mesencephalon; that constitutes an important step as it excludes the role for ascending, non-myelinated monoaminergic neurons as the MFB reward-relevant neurons.

Similar studies were performed in an attempt to characterize the reward-relevant neurons within the medial mesencephalon. The results showed that reward-relevant neurons in this region travel between the ventral tegmental area and the ventral central gray and that they have refractory periods that range from 0.3 to 1.0 msec (Bielajew et al., 1981; Rompré and Miliaressis, 1987; Boye and Rompré, 1996). The overlap between refractory period estimates for MFB and mesencephalic reward-relevant neurons as well as the mapping results suggest

that those neurons may be part of a common pathway travelling from the rostral brainstem to the basal forebrain.

Pharmacological characteristics of the reward-relevant pathway

As mentioned in the previous sections, a major objective following the discovery of BSR has been the identification of the reward-relevant neurons. To this end, a significant amount of research has been dedicated to locating those neurons in the CNS, to determine their trajectories and to determine their physiological characteristics. Another important step towards this aim has been the identification of the pharmacological properties of the reward-relevant neurons (i.e. the neurotransmitter(s) synthesized and released by these neurons). A summary of the recent findings is presented in this section.

Research aimed at identifying the pharmacological properties of BSR mainly involves central and/or systemic administration of drugs that alter the functions of the neurotransmitter; such alterations could be a functional potentiation/inhibition of the neurotransmitter or selective destruction of neurons that synthesized and release a given neurotransmitter. Since, drugs that could potentially alter reward could also alter performance, it is of prime importance that the method used to assess their effectiveness allows one to maximize the dissociation between reward and performance changes. Edmond and Gallistel (1977) were the first to use the curve shift method to investigate the effects of pharmacological agents on BSR. Based on an abundant literature implicating

catecholamine (DA and NE) in responding to BSR, they investigated the effects of a catecholamine (CA) synthesis inhibitor, α-methyl-p-tyrosine, on reward, and on performance. With the methods previously used, it was not clear whether the suppression of BSR following a reduction of CA neurotransmission was due to a decrease in rewarding effectiveness of the stimulation, to the sedative effect, or to impairment in motor performance. Edmonds and Gallistel's results clearly showed that at some stimulation sites within the lateral hypothalamus, α-methyl-p-tyrosine produced a selective attenuation of the rewarding effectiveness of the stimulation: Moreover, they showed that administration of L-dopa reversed the reward attenuation effect of α-methyl-p-tyrosine. These findings represent the first empirical evidence that the suppression of responding for BSR previously reported following a decrease in CA neurotransmission was due, at least in part, to a specific reward attenuation. A second step consisted of clarifying the contribution of each of the CAs, namely NE (NE) and dopamine (DA).

Noradrenaline. NE is a neurotransmitter found throughout the brain. NE cell bodies are located within the brain stem. Their projections originating from the locus coeruleus diffuse into several sub-cortical nuclei and cortical regions that do contain reward-relevant neurons. NE was the first CA to be investigated for its potential modulating effect on BSR. The early studies showed that disulfiram, a selective NE synthesis inhibitor, reduces the rate of responding for BSR (see Stein, 1980 for a review). Franklin (1978) used the Edmond and Gallistel's method of the curve-shift in the runway paradigm of self-stimulation to

investigate the effect of clonidine, a α-2 receptor agonist. When injected peripherally, clonidine reduced the rewarding effectiveness of the stimulation in the lateral hypothalamus and in the ventral tegmental area. The suppressant effect of clonidine was prevented by piperoxane, a α-2 antagonist. The suppressant effect of clonidine on reward was replicated by Gallistel and Freyd (1987) using the operant responding paradigm. These results suggest that α-2 noradrenergic receptor may be an important component of the reward-relevant circuitry and that noradrenaline acts through this receptor to attenuate reward. This hypothesis exclude a role for noradrenaline neurons as the directly stimulated reward-relevant neurons, an idea that is consistent with the conclusions drawn from anatomical and physiological data reviewed above.

Dopamine. In numerous studies DA has been shown to be implicated in BSR. Studies using indirect DA agonists and DA antagonists have consistently demonstrated that alterations in DA neurotransmission lead to alterations in BSR. For instance experiments performed in different laboratories have shown that several DA D-2 like receptor antagonists reduced the rewarding effectiveness of the stimulation (Franklin 1978; Stellar et al. 1983; Gallistel et al. 1984; Gallistel et al. 1987; Rompré and Boye 1996). In addition, systemic injection of SCH 23390, a selective D1-like receptor antagonist, was found to similarly reduce the rewarding effectiveness of the stimulation. (Nakajima and McKenzie 1986; Rompre and Bauco, 1990). A role for DA in BSR is also supported by results obtained with indirect DA agonists. In effect, drugs that increase synaptic DA

levels, like GBR-12909, a selective DA uptake blocker, amphetamine and cocaine were found to potentiate the rewarding effectiveness of the stimulation. (Rompre and Bauco 1990; Gallistel and Fryed, 1987; Maldonado-Irizarry et al. 1994; Bauco and Wise, 1997). Other pharmacological studies suggest that the DA neurons involved in BSR belong to the mesolimbic dopaminergic system. Ventral mesencephalic microinjections of drugs that increase and decrease mesolimbic dopaminergic impulse flow potentiate and attenuate BSR respectively (Rompré and Wise, 1989). Similarly, microinjections of drugs that potentiate and decrease DA neurotransmission in the ventral striatum were found to potentiate and attenuate respectively BSR (Rompré and Wise, 1989). Taken together, these results suggest that DA neurons that belong to the ascending mesostriatal system constitute an important component of the reward-relevant circuitry.

The empirical evidence supporting a role for mesostriatal DA neurons in BSR and several evidences raised interest for substances known to modulate their impulse flow. Two neuropeptides in particular, cholecystokinin and neurotensin, have been studied. The interest in these neuropeptides came first from anatomical studies showing that both neuropeptides co-exist with DA in the mesostriatal neurons. Secondly, neurotensin and cholecystokinin were found to alter mesostriatal impulses when they were injected directly into the ventral mesencephalon. The present work focuses on the role of neurotensin and its relevance to BSR.

Neurotensin. Neurotensin (NT) is a tridecapeptide of 13 amino acids (pGlu-Leu-Tyr-Asn-Lys-Pro-Agr-Pro-Tyr-Ile-Leu-OH) that was first isolated from the hypothalamus (Carraway and Leeman, 1976). It is present in the peripheral nervous system and the CNS and it meets all criteria for classification as a neurotransmitter (Carraway et al. 1982; Mai et al. 1987).

Peripherally, NT has been found to produce physiological effects principally on the digestive tract and on the cardio-vascular system such as hyperglycemia, smooth muscles contraction and inhibition of gastric acid secretion (Nemeroff, 1986).

Centrally, NT has been found mainly in those nuclei that belong to the limbic system. Large concentrations of NT were detected for instance in the amygdala, the striatum, the septum, the habenula, the anterodorsal nucleus of the thalamus, the bed nucleus of the stria terminalis, the hypothalamus, the ventral midbrain and the periaqueductal gray (Robert et al. 1982; Goedert et al. 1984; Moyse et al. 1987). Microinjections of NT into the brain (intracisternally or intracerebroventricularly) have been found to produce analgesia (Clineschmidt et al., 1979), hypothermia (Bissette et al., 1976; Nemeroff et al. 1979; Martin et al. 1988; Kalivas et al. 1982; Bissette et al. 1989), reduction in muscular tonus (Osbahar et al. 1979), alteration in food consumption (Luttinger et al. 1982), inhibition and excitation of locomotor activity (Nemeroff and al. 1977; Kalivas 1981) and changes in responding for BSR (Rompré, 1995).

At present, two NT receptors have been cloned: NTR1 and NTR2. NTR1 is a G-protein coupled receptors that displays a high affinity for NT; its activation has been linked to several biochemical events such as calcium influx, cyclic guanosine-monophosphate and phosphoinositol production (Canonico and al. 1985; Yamada and al.,1993). NTR2 displays a lower affinity for NT than NTR1; it was originally differentiated from NTR1 by its ability to bind an antihistaminic drug, levocabastine (Schotte and al. 1986). Just like NTR1, NTR2 belongs to a G-protein coupled receptors but to date no biochemical event has been linked to its activation; because of this, NTR2 has been considered as an acceptor site, a term referring to its ability to bind NT without inducing any physiological effect. NTR1 and NTR2 have differential affinities for the selective NT antagonists, SR-48692 and SR-142948a, NTR1 displaying the highest affinity with SR-48692 (Labbe-Jullie, 1995) and the same affinity than NTR2 for SR-142948a (Batancour and al., 1998). Both receptors are found in limbic regions mentioned above that contain a high concentration of NT; a peculiar characteristic of NTR2 is its expression by neural as well as non-neural cells (astrocytes) (Schotte et al. 1988).

The relevance of NT for BSR initially came from its close interaction with mesencephalic DA neurons. Hence, NT is found in high concentrations in the ventral mesencephalon and in most areas of the limbic system innervated by DA terminals (Kalivas, 1985; Querion, 1985). Autoradiographic and immunohistochemical studies have revealed NT receptors on DA cell bodies and

dendrites, and on mesostriatal DA terminals (Woulfe and Beaudet 1989; Beaudet and Woulfe 1992). In addition, NT is co-localized with DA in a sub-population of mesocortical neurons (Berger et al., 1992). Activation of mesencephalic NT receptors stimulates DA impulse flow and DA release, and stimulates DA-dependent locomotor activity (Faggin 1990; Laitien et al., 1990; Elliott et al., 1986). It was also found to potentiate BSR (Rompré et al., 1993). Activation of NT receptors in the prefrontal cortex also stimulates mesencephalic DA impulse flow (Rompré et al., 1998), an effect that can be explained by activation of an excitatory efferent input to DA neurons. Such a stimulant effect of NT on DA functions contrasts with its inhibitory effect when injected into the ventral striatum. In effect, several studies have shown that activation of ventral striatal NT receptors inhibits DA-dependent behaviors (Kalivas et al., 1984). These findings show that the modulation of DA functions by NT occurs at the cell body level as well as at the terminal levels and that the direction of this modulation is dependent on its site of action.

Previous studies have demonstrated that NT has rewarding effects on its own. Glimcher et al. (1982,1984,1987) showed that rats will self-administer NT into the ventral mesencephalon, and that after repeated microinjections of NT in this region the rats exhibited a conditioned-place preference. Furthermore, Rompré et al. (1993) reported that ventral mesencephalic microinjections of NT, and its active analogs, potentiated BSR. These findings are consistent with the reported activation of mesencephalic DA impulse flow by NT receptor activation.

In view of i) the role of mesencephalic DA neurons in BSR, ii) the modulation of mesencephalic DA functions by NT and iii) the potentation of BSR by central and ventral mesencephalic microinjections of NT, it can be hypothesized that NT is an important component of the reward-relevant circuitry. The following experiments were designed to test this hypothesis. We tested the effects of several doses of the two selective NT antagonists, SR-48692 and SR142948a on BSR using the curve-shift method.

Material and Methods

Subjects

Subjects were male Long-Evans rats (Charles River, St-Constant, Qué.) weighing between 320g and 450g at the time of surgery. They were housed individually within polyethylene cages, in a temperature- and humidity-controlled room, and maintained on a 12-hr light/dark cycle (light on at 6:00 and off at 18:00). They had constant access to food and water and were given one week habituation to the room upon their arrival.

Surgery

Each rat was first injected with 0.3 mg/kg (i.p.) of atropine methylnitrate to prevent accumulation of mucous in the bronchi. Fifteen minutes after the atropine injection, the rat was anaesthetized with sodium pentobarbital (Somnotol, 65 mg/kg, ip) and secured to a stereotaxic frame. Using standard surgical procedures, a moveable stimulating electrode (Kinetrods, model SME-01) made

from a stainless-steel wire (0.25 mm diameter) insulated to the tip with Epoxy-Lite was implanted into the medial posterior mesencephalon. The stereotaxic coordinates were 7.6 mm posterior to bregma, 0 mm lateral to the sagittal suture and 6.8 mm below the surface of the skull (Paxinos and Watson, 1986). An uninsulated stainless-steel wire was wrapped around five miniature stainless-steel screws threaded into the cranium; it served as the inactive electrode. The electrode assembly was secured to the cranium with dental acrylic. Penicillin G (Penlong XL, 15,000 U/0.1 ml, I.M.) was administered as a prophylatic treatment for infection.

Drug Preparation

SR-48692 and SR-148942a were initially mixed in 2 drops of Tween-80 suspended in 20 ml of distilled water. The vehicle consisted of 20 ml of distilled water. Each solution was sonicated and stirred for several minutes just before the injection. The drug and vehicle solution was administered intraperitoneally 30 min before testing in a volume of 1 ml/kg (40, 80, and 160 μg/kg) or 2 ml/kg (3000 μg/kg).

Training procedures

One week (at least) after the surgery, the rats were screened for BSR behaviour using a standard shaping procedure. They were placed into operant boxes made from plywood and Plexiglas; each box contained a lever that protruded 2-3 cm from one side of the wall. Each operant box was placed into a

larger insulated wooden box with a Plexiglas door in the front of the box; an electrical fan located at the back of the insulated box insured proper ventilation. Electrodes were connected to the stimulator by a flexible lead attached to a swivel allowing the animal to move freely. Depression of the lever triggered a constant-current stimulator (Mundl, 1980) that delivered a 200 ms train of cathodal, rectangular pulses (0.1 ms in duration) through the moveable stimulation electrode.

Initially, the current intensity was set at 400 μA. The effect of the stimulation on the behaviour of the rat was determined by delivering several non-contingent trains of pulses as the stimulation frequency was varied from 25 to 100 Hz. If the stimulation elicited an aversive effect, the electrode was lowered by 400 μm using a procedure previously described (Rompré and Miliaressis, 1985), and the rat was screened again 24 h later. However, If the stimulation elicited behavioural responses generally predictive of a rewarding effect such as sniffing and exploratory behaviours, (Rompré and Miliaressis, 1980), the experimenter attempted to train the rat to press on the lever to self-administer the stimulation. The current intensity and stimulation frequency were increased up to 800 μA and 100 Hz respectively until the rats lever press. In the event that the rat did not lever press after 30 min of shaping, the electrode was lowered by 400 μm and the rat was tested again the next day. Rats that failed to self-stimulate after the electrode had been lowered four times (i.e. 1.6 mm), were discarded from the study.

Responding rats were first allowed to self-stimulate during 3 consecutive daily sessions. Each session consisted of four 15 min BSR periods separated by a 1 min period of rest; the stimulation frequency was alternated between 75 and 85 Hz (from one period to the other), and the current intensity was adjusted to induce constant responding. The beginning of each BSR period was signalled by delivery of 10 trains of priming stimulation at a rate of 1 train/sec. All parameters of the stimulation, and of the BSR period, were controlled by a microcomputer that also recorded the number of lever presses. After this initial training phase, the rats were trained to lever-press during several 45 sec discrete trials that were separated by a 25 sec inter-trial interval during which the lever was disconnected. Five trains of stimulation delivered at a rate of one train/sec announced the beginning of each trial. Using this procedure, the rats were trained for three consecutive before which the second training phase began. This second phase consisted of determining four functions that relate the rate of lever pressing to the stimulation frequency (R-F function). The stimulation frequency was initially set at 115 Hz and was decreased by approximately 10% on every subsequent 45 sec trial until it reached 25 Hz; the current intensity was adjusted to induce responding near a frequency of 50 Hz. A frequency threshold, defined as the frequency required to produce a rate of lever pressing equal to 50% of the maximal rate (M50), was calculated from each of the R-F functions. Responding was considered stable when the M50 index varied by less than 0.1 log unit for three consecutive days.

Drug Testing

Once stable responding was established, the rats were divided into five groups and drug testing began. A drug test consisted first of determining three R-F functions using the procedure just described. The rats were then removed from the operant box and injected IP with one of 4 doses of SR-48692 (40 μg/kg, group 1; 80 μg/kg, group 2; 160 μg/kg, group 3 and 3000 μg/kg, group 4), or its vehicle (group 5) and placed back into the operant boxes. Thirty minutes after the injection, five new R-F functions were determined during a test period that lasted approximately 90 min. A post-test consisting of three R-F function determinations was performed 24h later. The range of SR-48692 doses tested was chosen on the basis of previous studies demonstrating their effectiveness at blocking the behavioural and physiological effects of neurotensin (see Gully et al., 1993).

As a pilot study, four rats from group 4, 1 rat from group 2, and 2 rats from group 5 were also tested with three effective doses (40, 160 μg/kg, 640 μg/kg) of the new selective neurotensin antagonist, SR-142948a (see Gully et al., 1997) using identical procedures; at least seven days separated each drug test. Rats all the other rats were drug tested only once.

Histology

At the end of the study, the rats were deeply anaesthetised with sodium pentobarbital (65 mg/kg, ip), or urethane (1.2 g/kg, ip), and the stimulation site was marked by passing a direct anodal current of 100 μA for 20sec through it.

The moveable electrode was then removed and the circulatory system was perfused with physiological saline (0.9%) followed by a 10% formalin solution. The brain was removed from the skull and placed in a 10% formalin solution containing 3% of potassium ferricyanide, 3% potassium ferrocyanide and 0.5% of trichloroacetic acid for 24h. The brains were rinsed thoroughly within 10% formalin solution for several days, and were subsequently sliced in 40 μm thick sections with a microtome. The brain sections were mounted on glass slides, dried for at least a week, and then stained with a thionine-formalin solution. The location of the stimulation sites were determined using light microscopic examination by two investigators independently.

Data Analysis

To assess whether the drug altered the parallelism of the R-F functions, two frequency thresholds were first calculated, M20 and M60. The thresholds were then expressed as percentage of pre-injection value. Statistical differences between the two indexes was determined using paired T-tests at a significance level of 0.05. Any difference between the two indexes would be considered to reflect a change in the slope of the R-F functions.

The statistical significance of the effects of the drugs on the rewarding effectiveness of the stimulation and on the maximal rates of responding was determined using the following procedure. Frequency thresholds were first calculated (M50 and Theta0) for the last 2 R-F functions determined before the

injection, and averaged them for each rat, in each group. Then, for the 5 R-F functions determined after the injection, for each rat in each group, thresholds and maximal rates were calculated and expressed as the percentage pre-injection value. Statistical significance was determined using a two-way ANOVA for repeated measures on the time factor. The significance level was set at 0.05.

Results

Of the 67 rats initially prepared for the experiment, 45 completed the study. Sixteen rats were excluded either because they could not be trained to self-stimulate, they did not reliably respond during the training phase, or they stopped responding during the course of the experiment. The complete set of data obtained from six other rats was discarded because of technical problems that occurred during the test period.

Histological Results

The location of the stimulation sites of the rats that were assigned to each group are presented in Figs. 1 to 5. The drawings represent coronal sections from Paxinos and Watson's atlas (1986) of the rat brain.

The stimulation sites of the rats in the vehicle-control group were located within or below the ventral central gray, at the anterior-posterior levels between 7.3 and 7.6 mm posterior to bregma (Fig. 1). Two sites were located near the

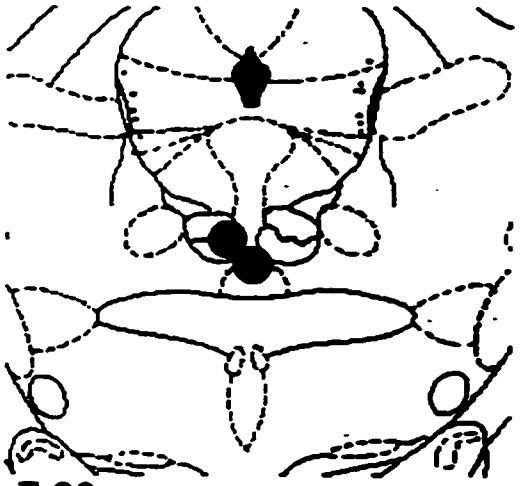
medial longitudinal fasciculus, six in the decussation of the superior cerebellar peduncle, and two in the dorsal raphe nucleus.

The stimulation sites of the rats in the group tested with 40 μg/kg of SR-48692 were located between 7.3 and 8.0 mm posterior to bregma (Fig. 2). One site was located near the medial longitudinal fasciculus, one in the caudal linear nucleus raphe, and six in the dorsal raphe.

The stimulation sites of the rats in the group tested with 80 μg/kg of SR-48692 were located between 6.8 and 8.3 mm posterior to bregma (Fig. 3). Three sites were located in the ventral central gray, two in the dorsal tegmental decussation, and four in the dorsal raphe nucleus. The stimulation sites of the rats in the group tested with 160 μg/kg of SR-48692 were located between 7.3 and 8.3 mm posterior to bregma (Fig. 4). One site was in the decussation of the superior cerebellar peduncle, one in the medial longitudinal fasciculus, one in the caudal linear nucleus raphe, and six in the dorsal raphe. The stimulation sites of the rats in the group tested with 3000 μg/kg of SR-48692 were located between 6.3 and 7.8 mm posterior to bregma (Fig. 5). Four sites were located in dorsal raphe, three in the decussation of the superior cerebellar peduncle and two in the caudal linear nucleus raphe.

Figure 1. Histological representation of the stimulation sites for animals included in the vehicle-control group (n= 10). Drawings are from the Paxinos and Watson's atlas of the rat brain (1997). Each circle represents the stimulation site for one or more rats.

SR-48692
Vehicle



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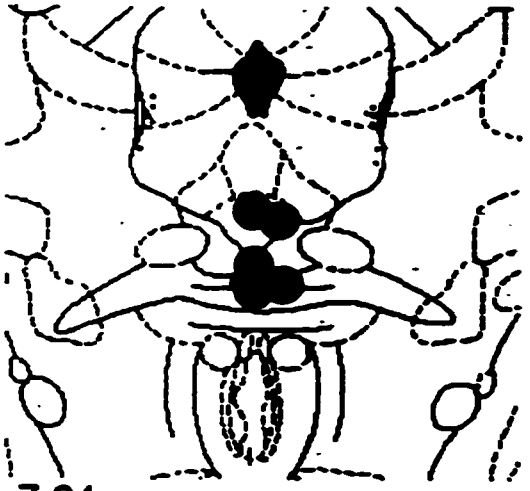
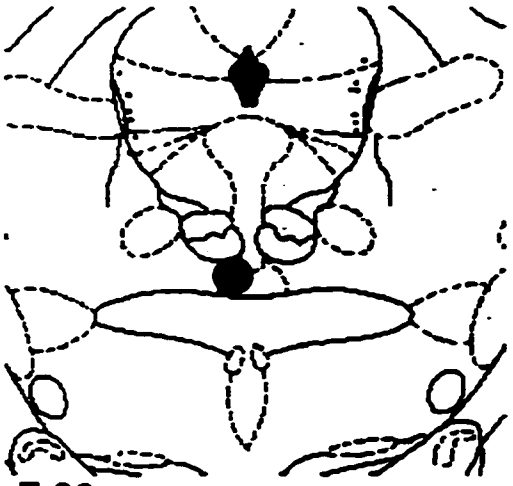
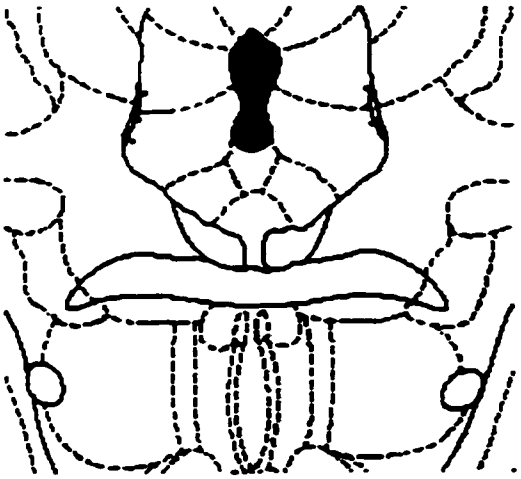


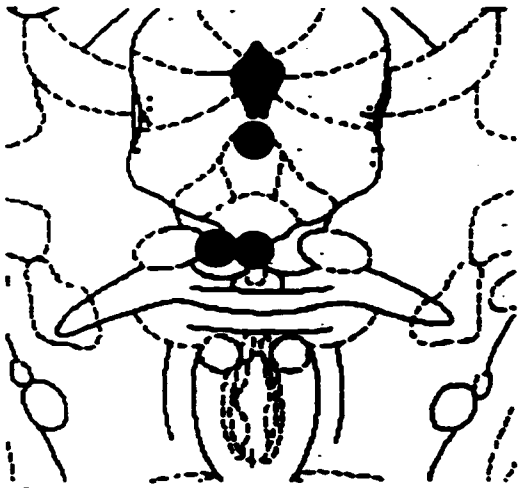
-7.64

Figure 2. Histological representation of the stimulation sites for animals that received 40 μg/kg of SR-48692 (n= 8).

SR-48692
40 µg/kg







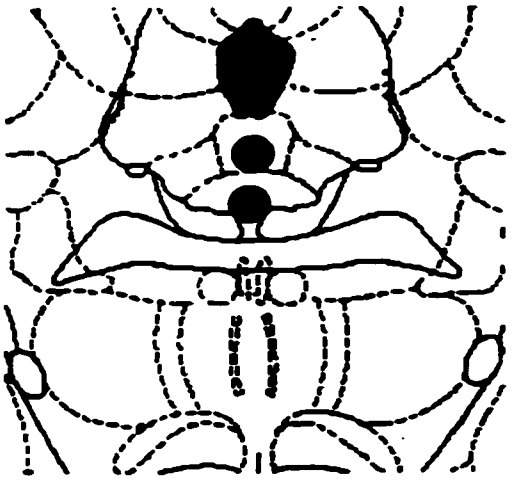
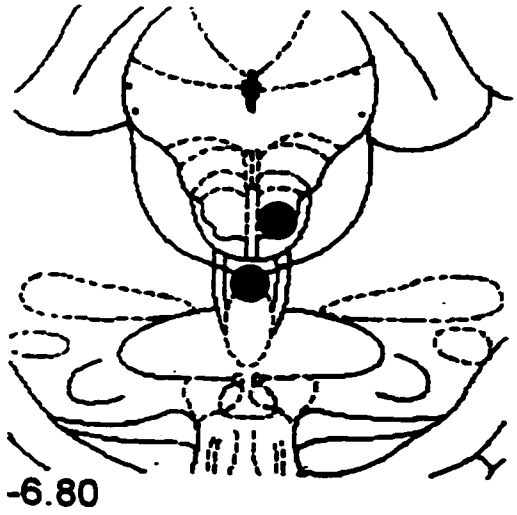
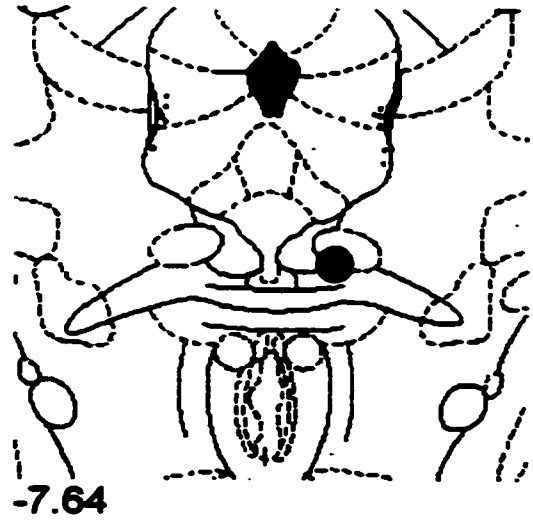
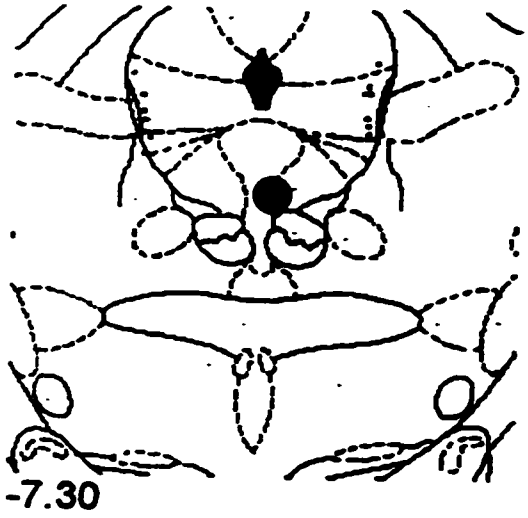


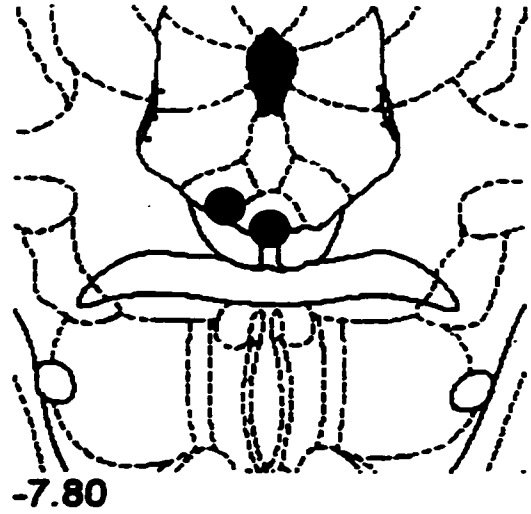
Figure 3. Histological representation of the stimulation sites for animals that received 80 μg/kg of SR-48692 (n= 9).

SR-48692
80 µg/kg









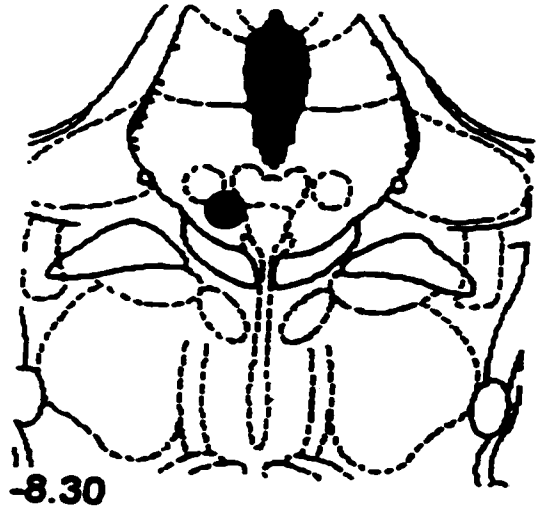
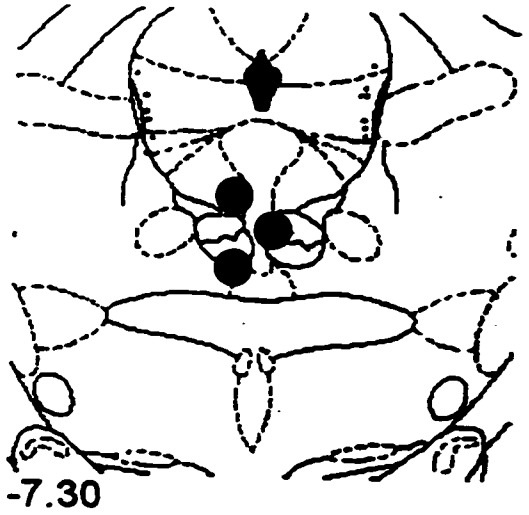


Figure 4. Histological representation of the stimulation sites for animals that received 160 μg/kg of SR-48692 (n= 9).

SR-48692
160 µg/kg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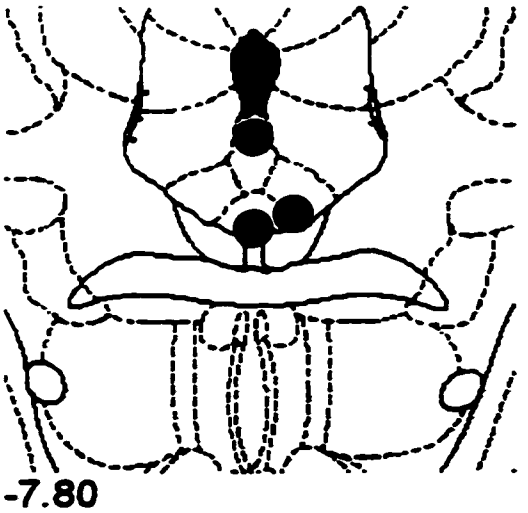
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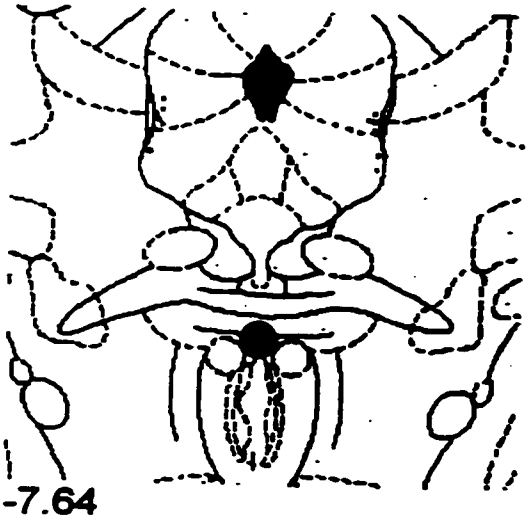
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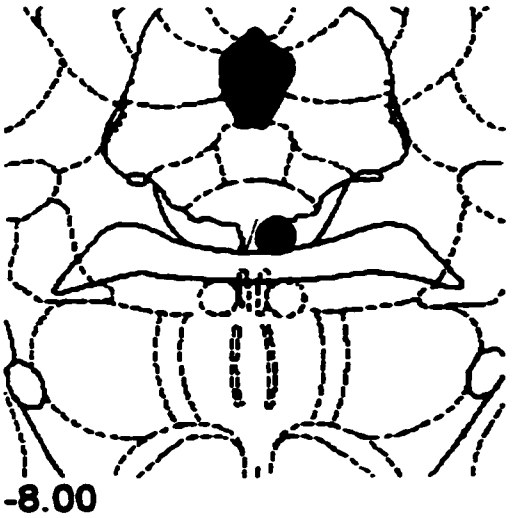
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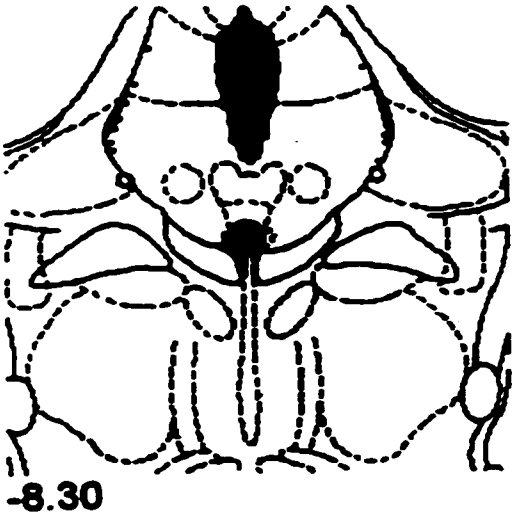
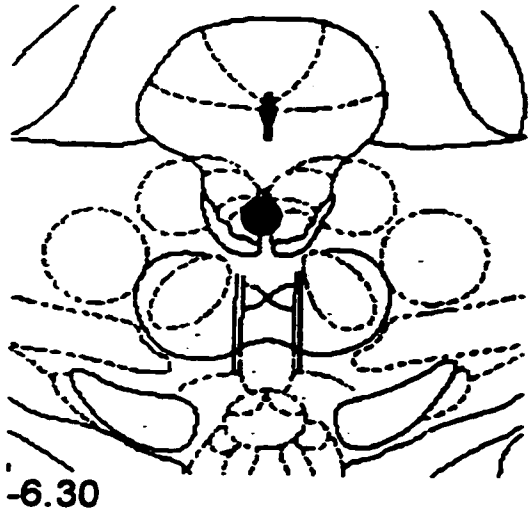
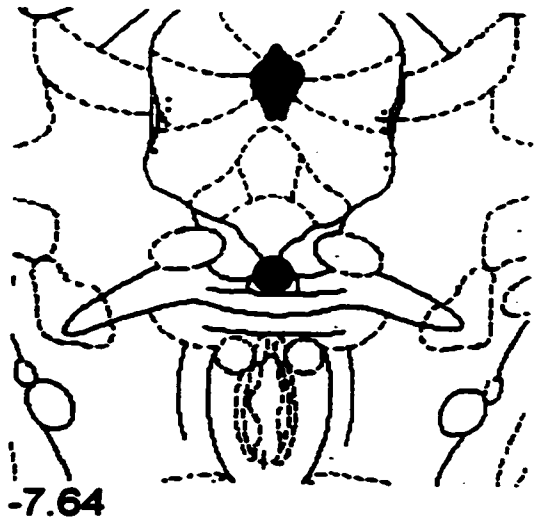
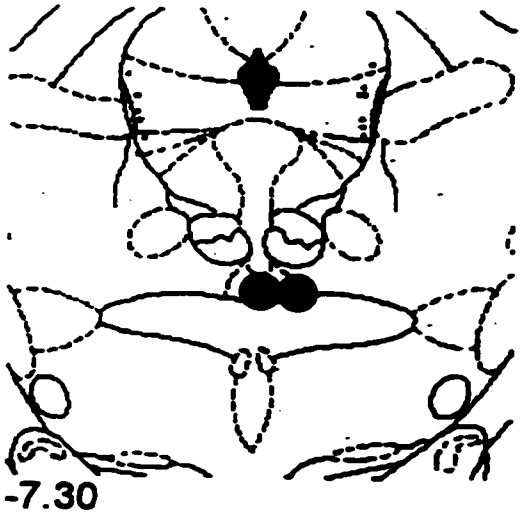

-8.30

Figure 5. Histological representation of the stimulation sites for animals that received 3000 μg/kg of SR-48692 (n= 9).

SR-48692
3000 µg/kg







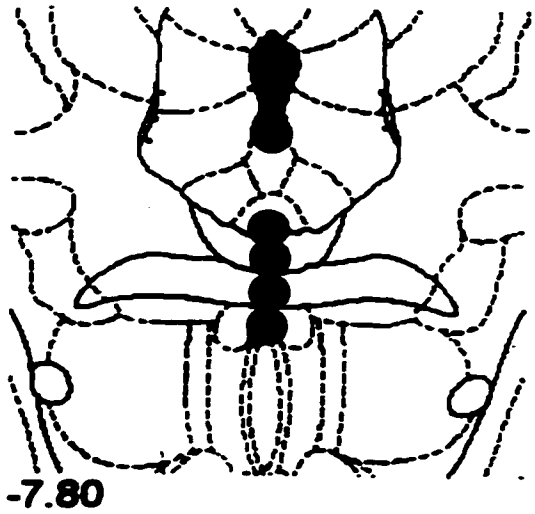
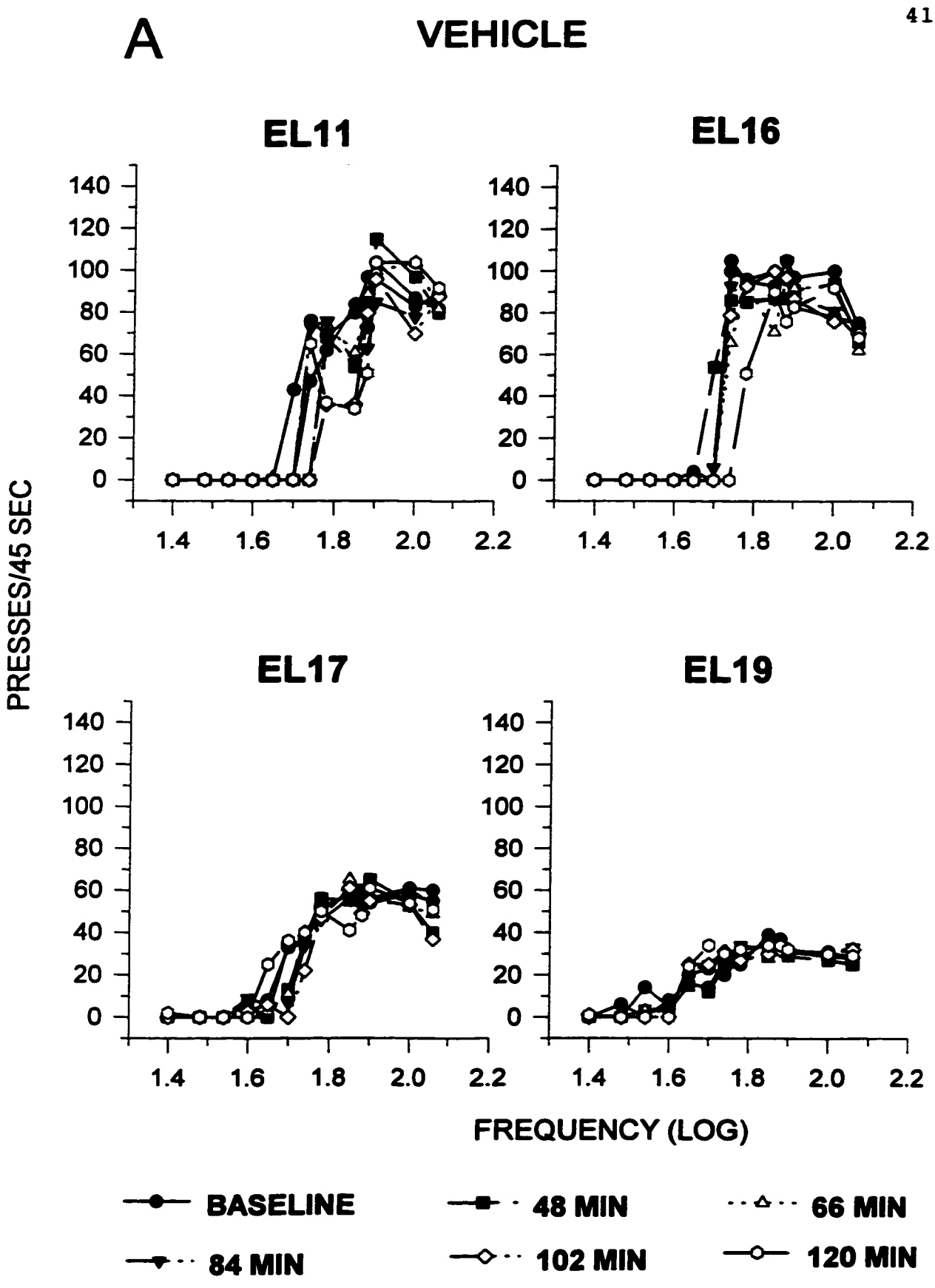
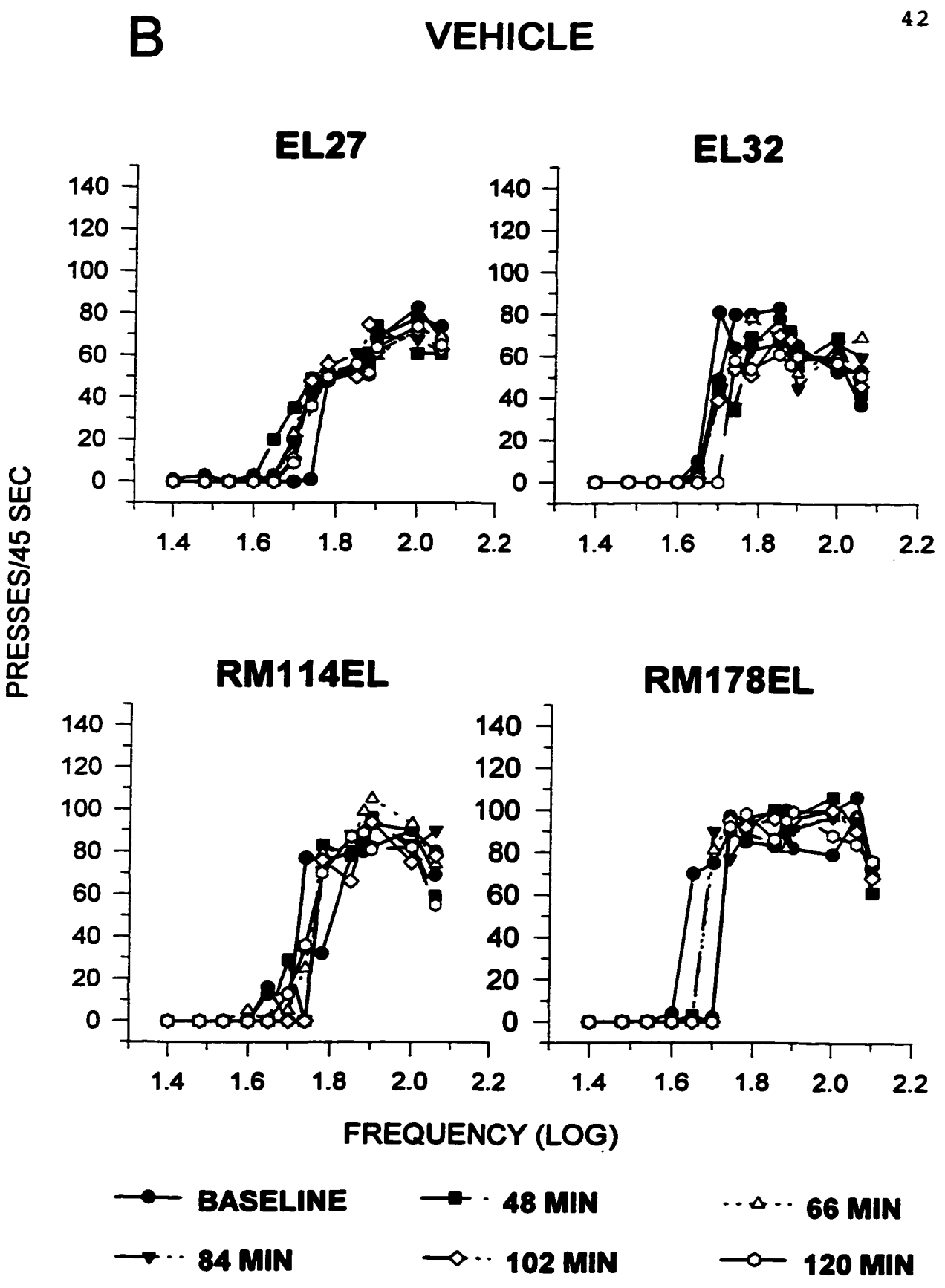


Figure 6 A, B, C. Rates of bar pressing as a function of the stimulation frequency (in log) obtained before, and at 48, 66, 84, 102 and 120 min after, injection of the vehicle.


A
VEHICLE
EL11
EL16
EL17
EL19
PRESSES/45 SEC
FREQUENCY (LOG)
140
120
100
80
60
40
20
0
1.4
1.6
1.8
2.0
2.2
BASELINE
48 MIN
66 MIN
84 MIN
102 MIN
120 MIN

B


VEHICLE
EL27
EL32
RM114EL
RM178EL
PRESSES/45 SEC
FREQUENCY (LOG)
BASELINE
48 MIN
66 MIN
84 MIN
102 MIN
120 MIN

C

VEHICLE

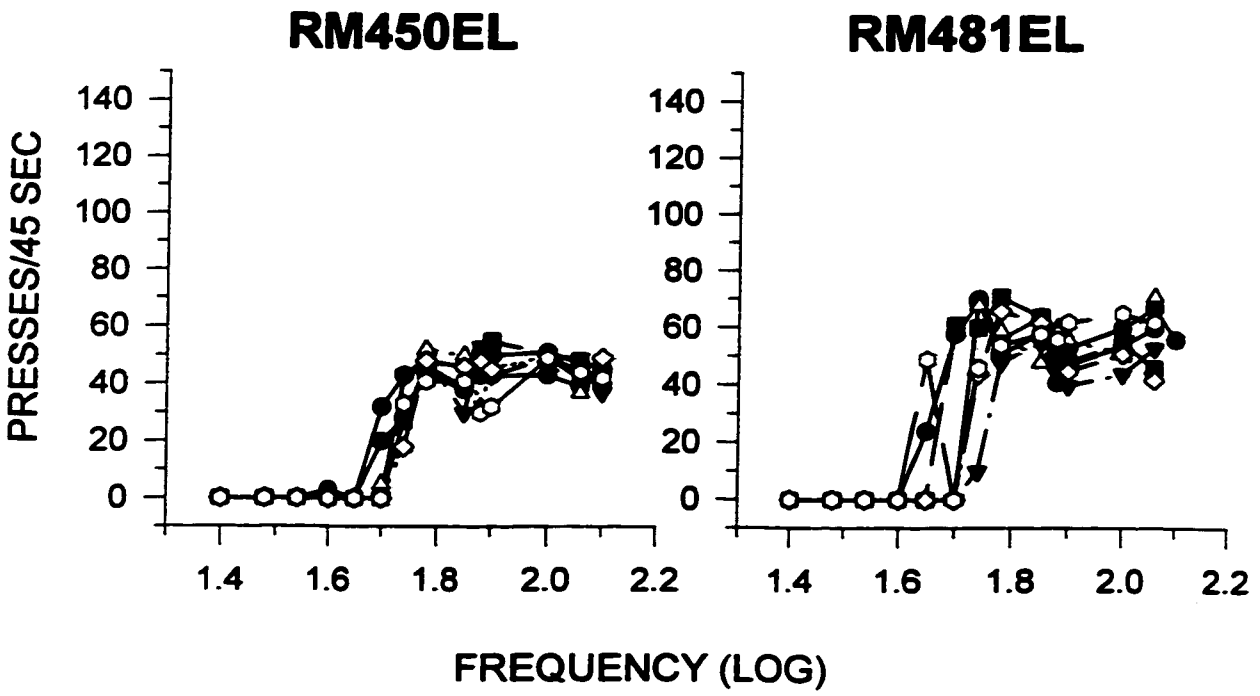
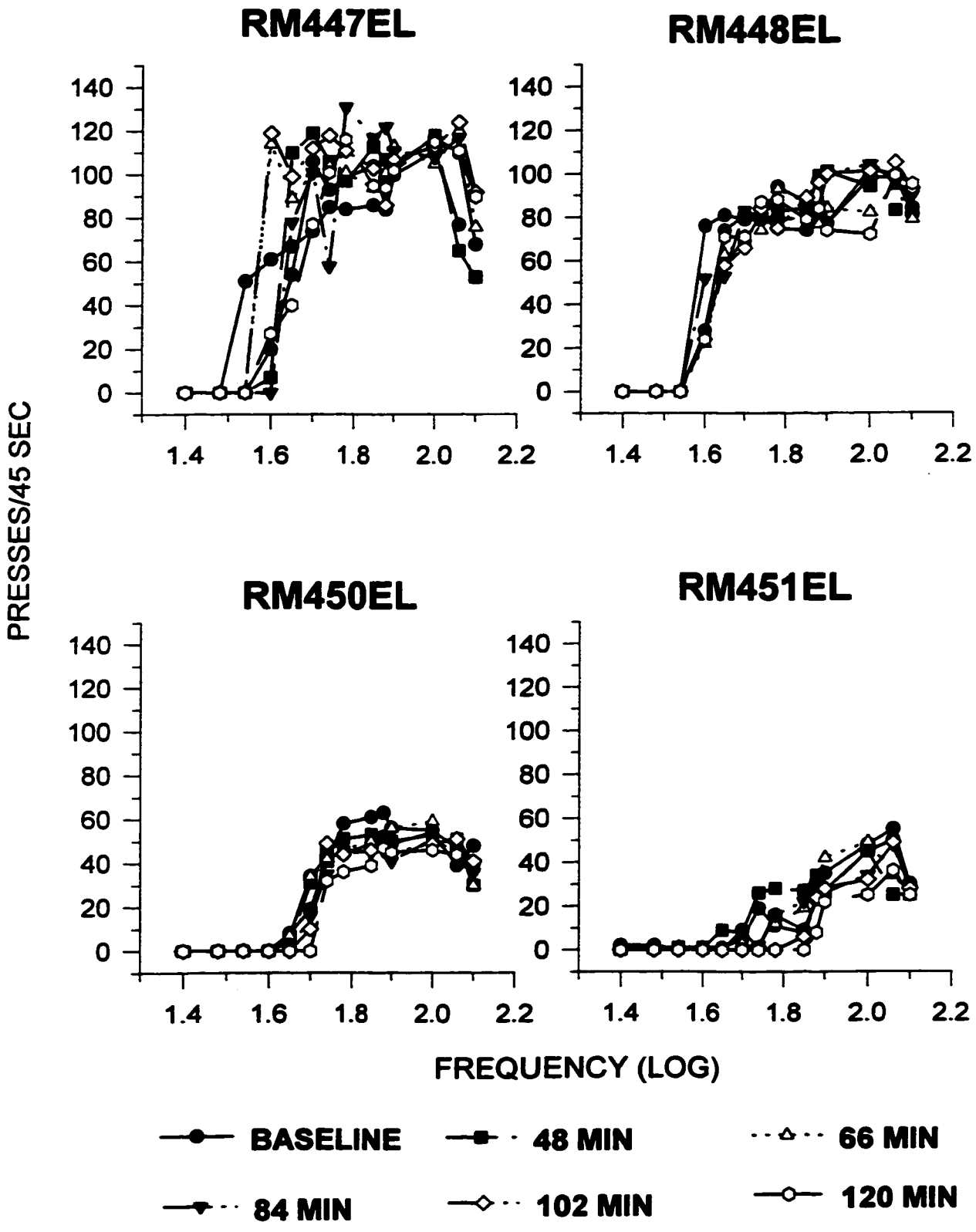

RM450EL
RM481EL
PRESSES/45 SEC
140
120
100
80
60
40
20
0
1.4
1.6
1.8
2.0
2.2
FREQUENCY (LOG)
BASELINE
48 MIN
66 MIN
84 MIN
102 MIN
120 MIN

Figure 7 A, B. Rates of bar pressing as a function of the stimulation frequency (in log) obtained before, and at 48, 66, 84, 102 and 120 min after, injection of 40 µg/kg of SR-48692.

A


40 µg/kg SR-142948a
RM447EL
RM448EL
RM450EL
RM451EL
PRESSES/45 SEC
FREQUENCY (LOG)
BASELINE
48 MIN
66 MIN
84 MIN
102 MIN
120 MIN

B 40 µg/kg SR-142948a

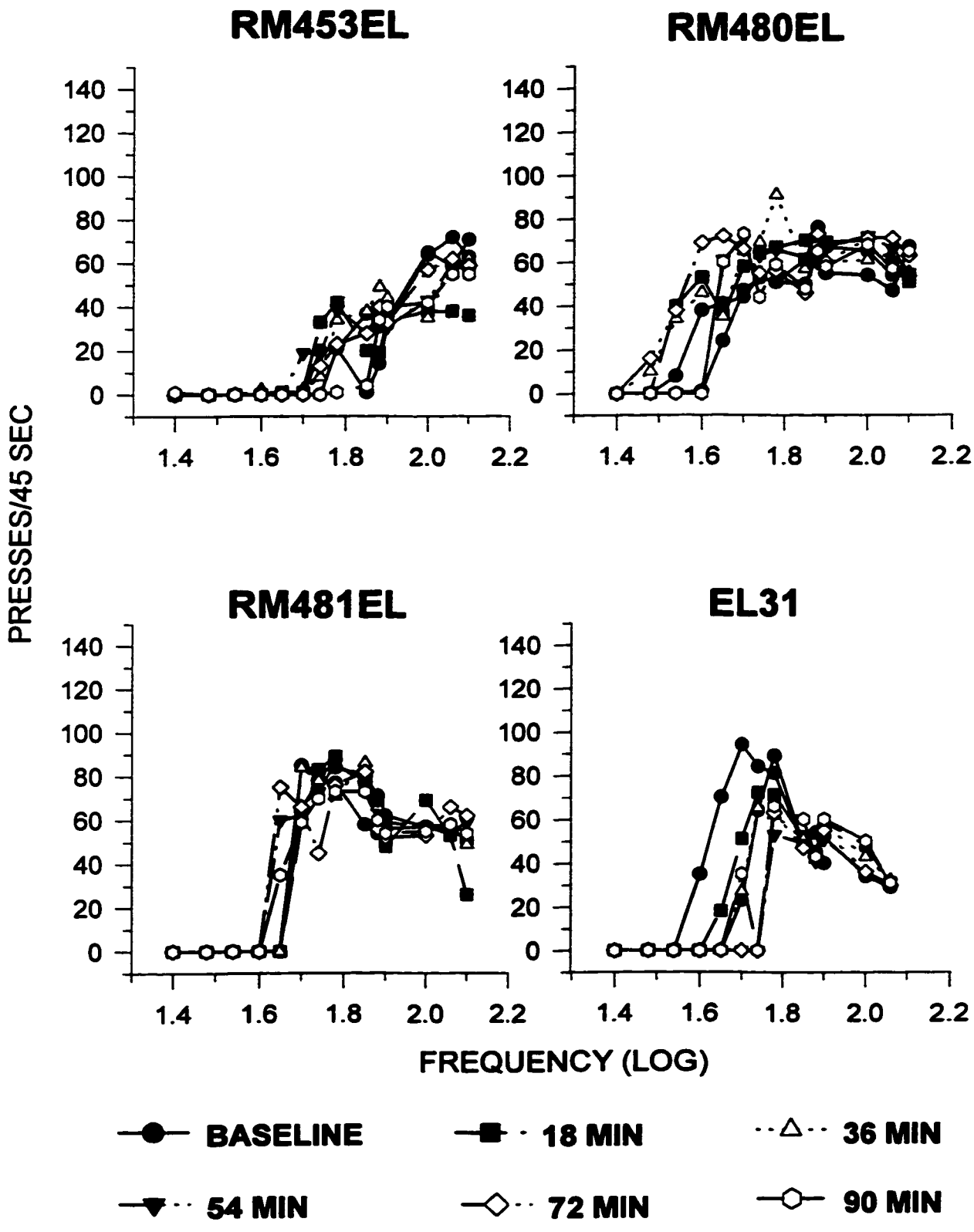
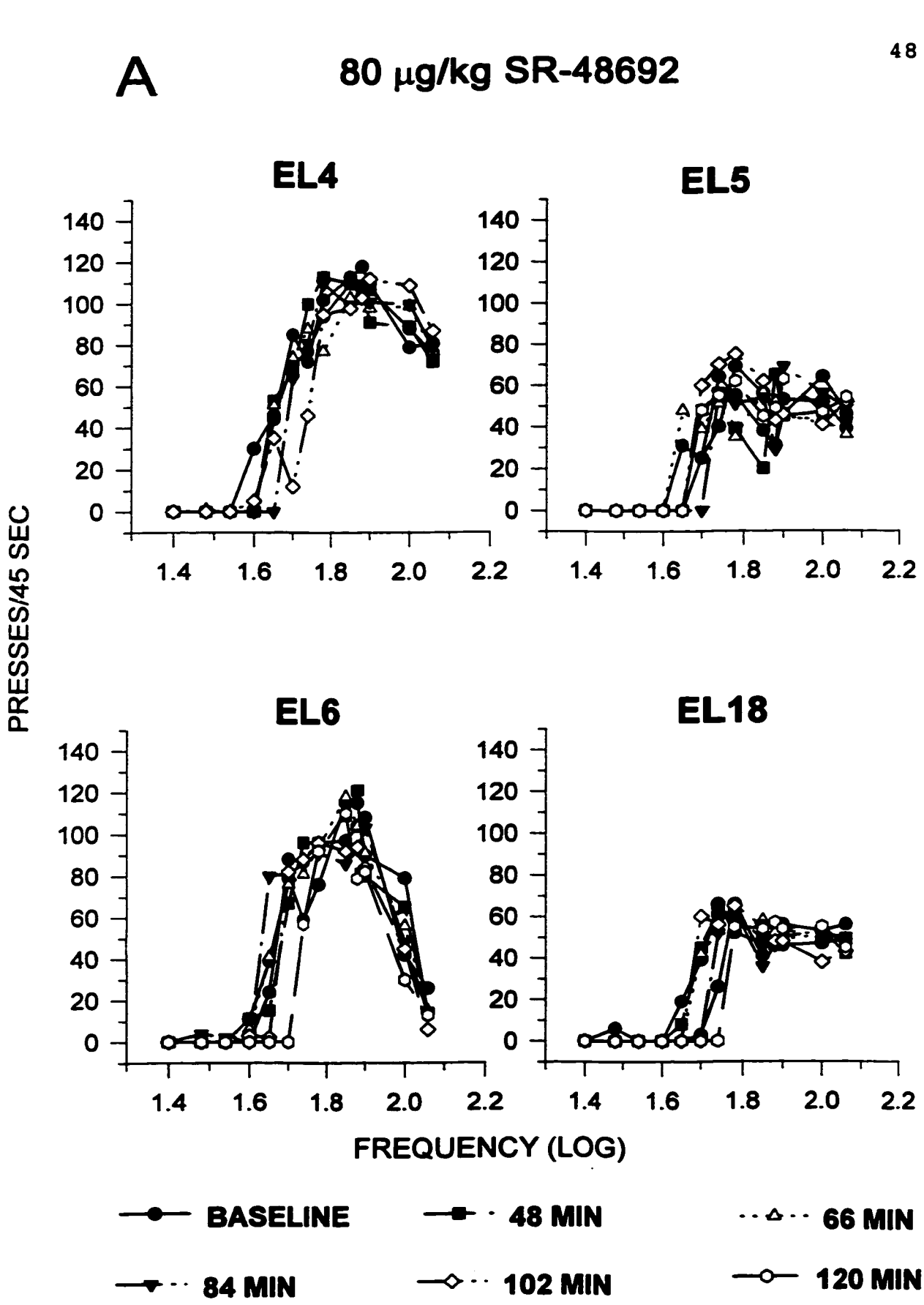
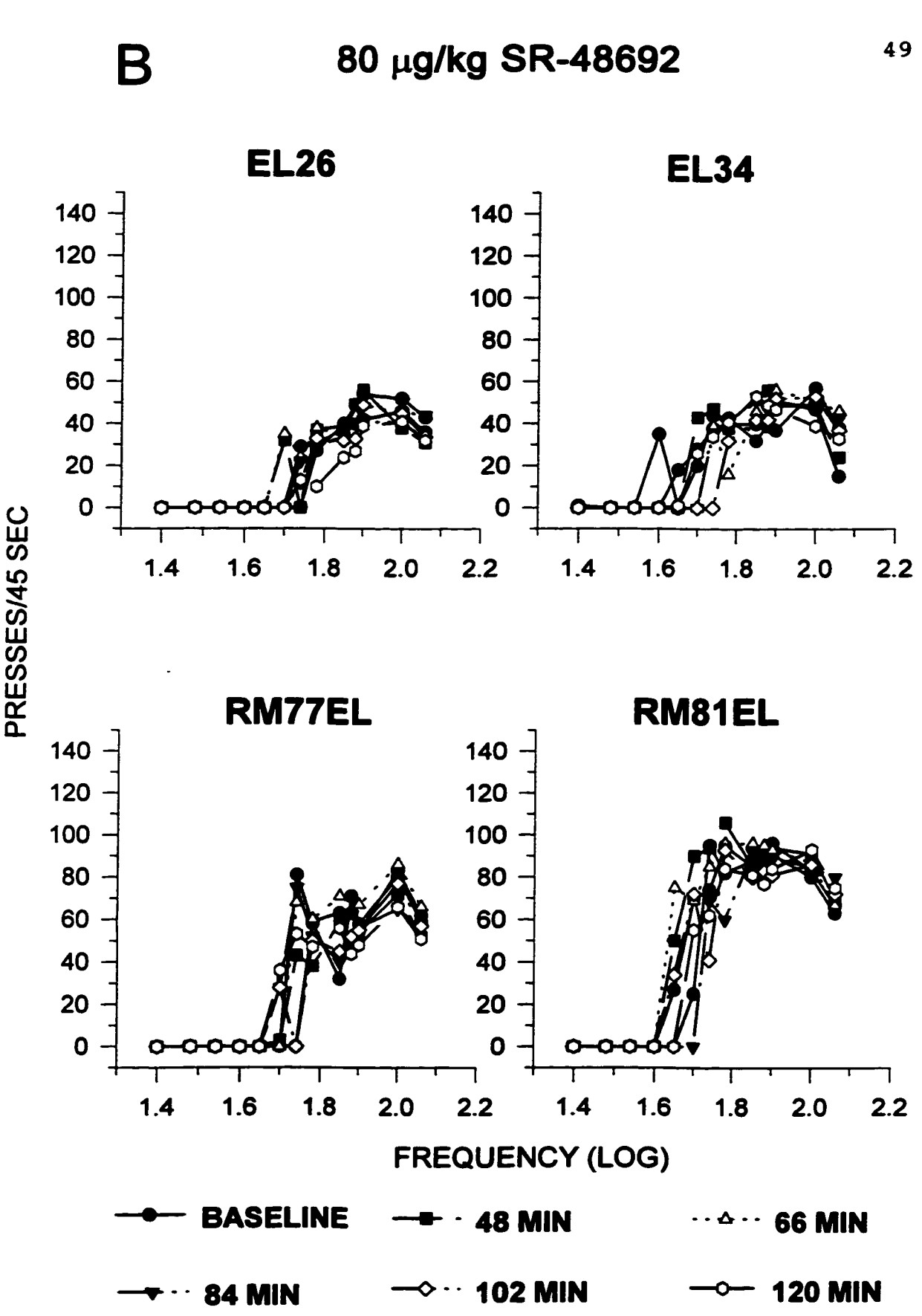


Figure 8 A, B, C. Rates of bar pressing as a function of the stimulation frequency (in log) obtained before, and at 48, 66, 84, 102 and 120 min after, injection of 80 μg/kg of SR-48692

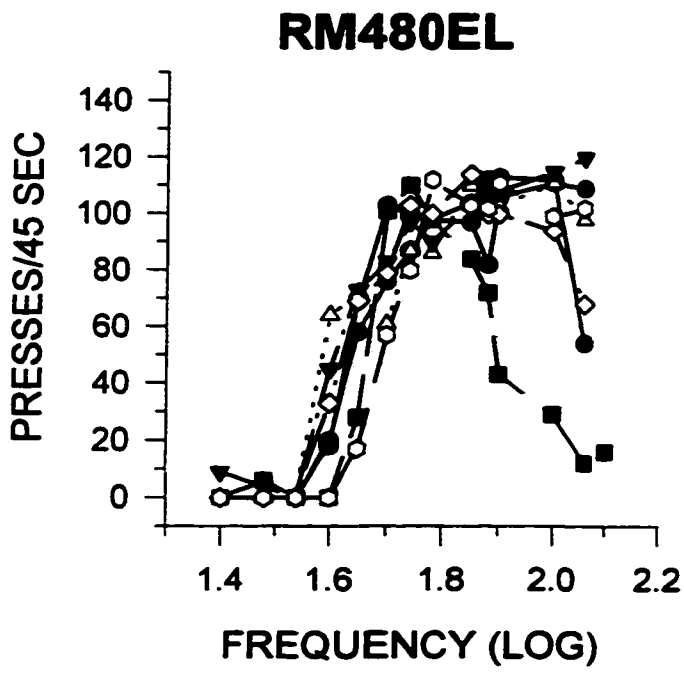
A


80 µg/kg SR-48692
EL4
EL5
EL6
EL18
PRESSES/45 SEC
FREQUENCY (LOG)
BASELINE
48 MIN
66 MIN
84 MIN
102 MIN
120 MIN

B


80 µg/kg SR-48692
EL26
EL34
RM77EL
RM81EL
PRESSES/45 SEC
FREQUENCY (LOG)
BASELINE
48 MIN
66 MIN
84 MIN
102 MIN
120 MIN

C 80 µg/kg SR-48692



BASELINE
48 MIN
66 MIN
84 MIN
102 MIN
120 MIN

SR-48692

Rate-Frequency Functions. The R-F functions obtained before, and at five time periods after, vehicle injection are presented in Fig. 6a, 6b and 6c. Several observations can be made. First, as expected, the R-F functions have a sigmoïdal shape, a flat line at low frequencies followed by an ascending portion and a plateau. Second, the maximal rates of bar presses represented by the plateau (>1.8 log units) varied little before and after the injection. Third, the position of the R-F function on the x-axis is also relatively stable, varying at most by 0.1 log unit (i.e rats EL11, RM481EL). The visual observation of the relative stability of the shape of the R-F functions, and of their position on the x-axis, suggests that the vehicle injection had weak effects on the rewarding effectiveness of the stimulation and on the ability of the animal to produce an operant response.

The rate-frequency functions obtained before, and at five time periods after, injection of 40 μg/kg of SR-48692 are presented in Fig. 7a and 7b. At this dose, SR-48692 had weak effects on the R-F functions. For most of the rats, maximal rates of responding changed little as the position of the functions on the x-axis. In the case of rat EL31, however, noticeable rightward shifts were observed, and a peak occurred at 102 min after the injection (0.2 log unit).

Figure 8 illustrates the effects of an injection of 80 μg/kg of SR-48692 on the R-F functions for the nine rats included in this group. As seen previously at a

lower dose, injection of 80 μg/kg of SR-48692 had little effect on the shape of the R-F functions. The maximal rates of responding change very little as the position of the functions on the x-axis. For one rat (RM480EL), however, we observed a large suppression of rates of bar pressing for high stimulation frequency at 48 min after the injection.

Figure 9 illustrates the effects of an injection of 160 μg/kg of SR-48692 on the R-F functions for the nine rats included in this group. Again, injection of this dose had little effect on the shape and on the position of the R-F functions on the x-axis. The largest change, a rightward shift, was observed 84 min after the injection in rat EL7. It is noteworthy that this shift was not accompanied by any noticeable changes in maximal rates.

Figure 10 illustrates the effects of an injection of 3000 μg/kg of SR-48692 on the R-F functions for the nine rats included in this group. As was the case for the three other doses tested, injections of SR-48692 at this dose level had little effect on the shape of the R-F functions. A larger variability across rats was observed, however, for the position of the R-F function on the x-axis. In the case of rat RM358EL, we observed very small lateral shifts while in the case of rat RM447EL, injections of SR-48692 produced a 0.2 log units rightward shift.

Maximal Rates and Reward Threshold. Figure 11 (top panel) shows the changes in maximal rates of responding (expressed in percentage pre-injection)

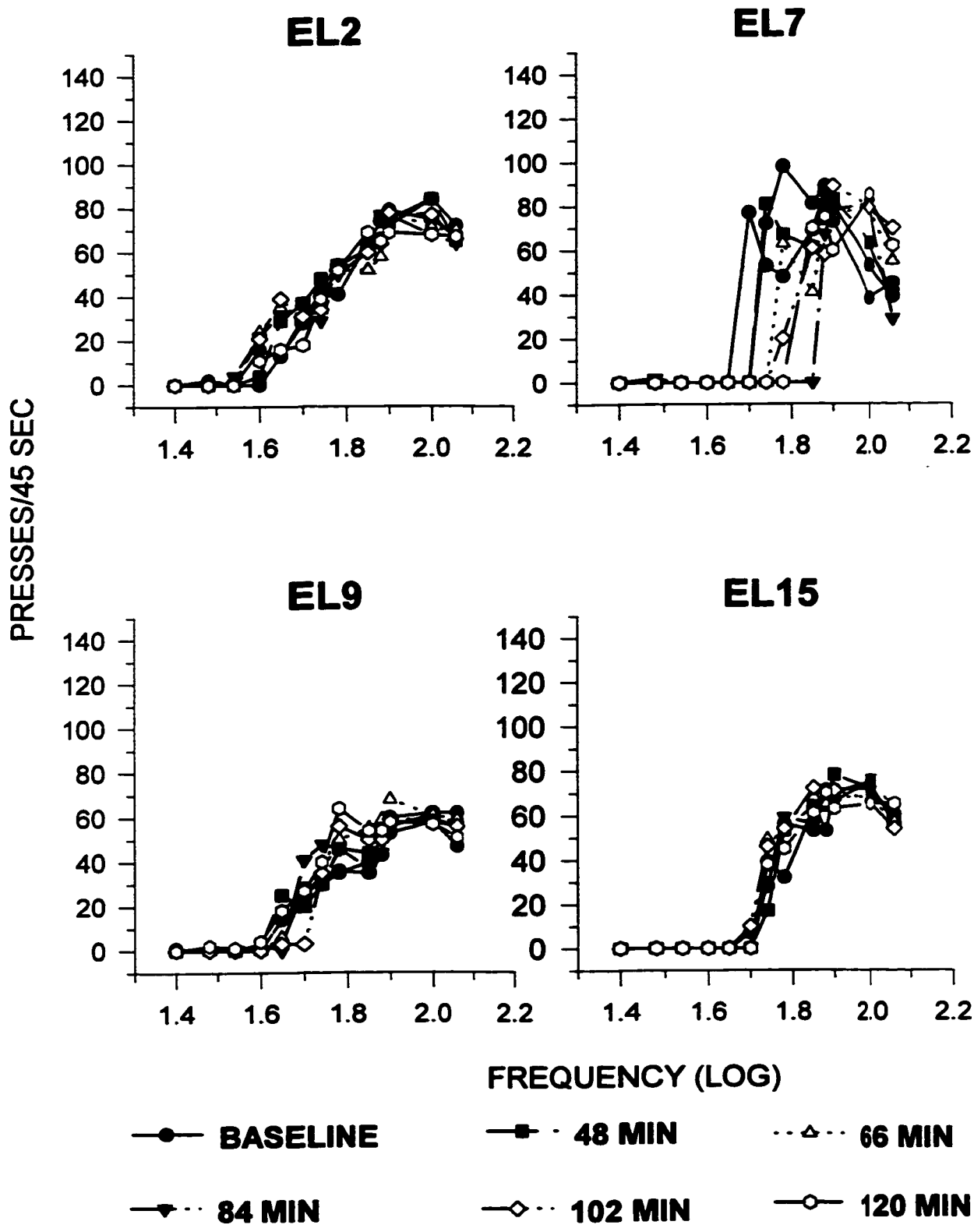
as a function of time after the injection of 40 (n= 8), 80 (n=9), 160 (n= 9) and 3000 (n= 9) μg/kg of SR-48692, or its vehicle (n= 10). At the lowest dose, SR-48692 produced a small decrease in maximal rates that peaked between 100 and 120 min after the injection. A similar decrease was observed after vehicle injection. The ANOVA revealed no significant effect of dose nor of time, and no significant time x dose interaction ($P > 0.05$). ($F(4,40) = 1.95$, $p = .12$ for dose; $F(4,160) = 1.95$, $p = .11$ for time; $F(16,160) = 1.16$, $p = .30$ for interaction dose x time).

Changes in reward thresholds (expressed in percentage pre-injection) as a function of time after the injection of 40, 80, 160 and 3000 μg/kg of SR-48692, or its vehicle, are shown in the bottom panel of Fig. 11. It can be seen that relative threshold values measured after the injection of each of the four doses of SR-48692 did not differ from those measured after vehicle injection. Hence, The ANOVA revealed no significant effect of dose, nor of time, and no signification time x dose interaction ($P > 0.05$). ($F(4,40) = 1.73$, $p = .16$ for dose; $F(4,160) = 1.59$, $p = .18$ for time; $F(16,160) = .89$, $p = .58$ for interaction dose x time).

Slope of the R-F functions. In order to determine whether the administration of SR-48692, or its vehicle, altered the slopes of the R-F functions, a statistical comparison of the changes in reward threshold was performed using two behavioural criteria: M20 and M60, the stimulation frequency required to produce rates of responding equal to 20% and 60% of the maximal rates, respectively.

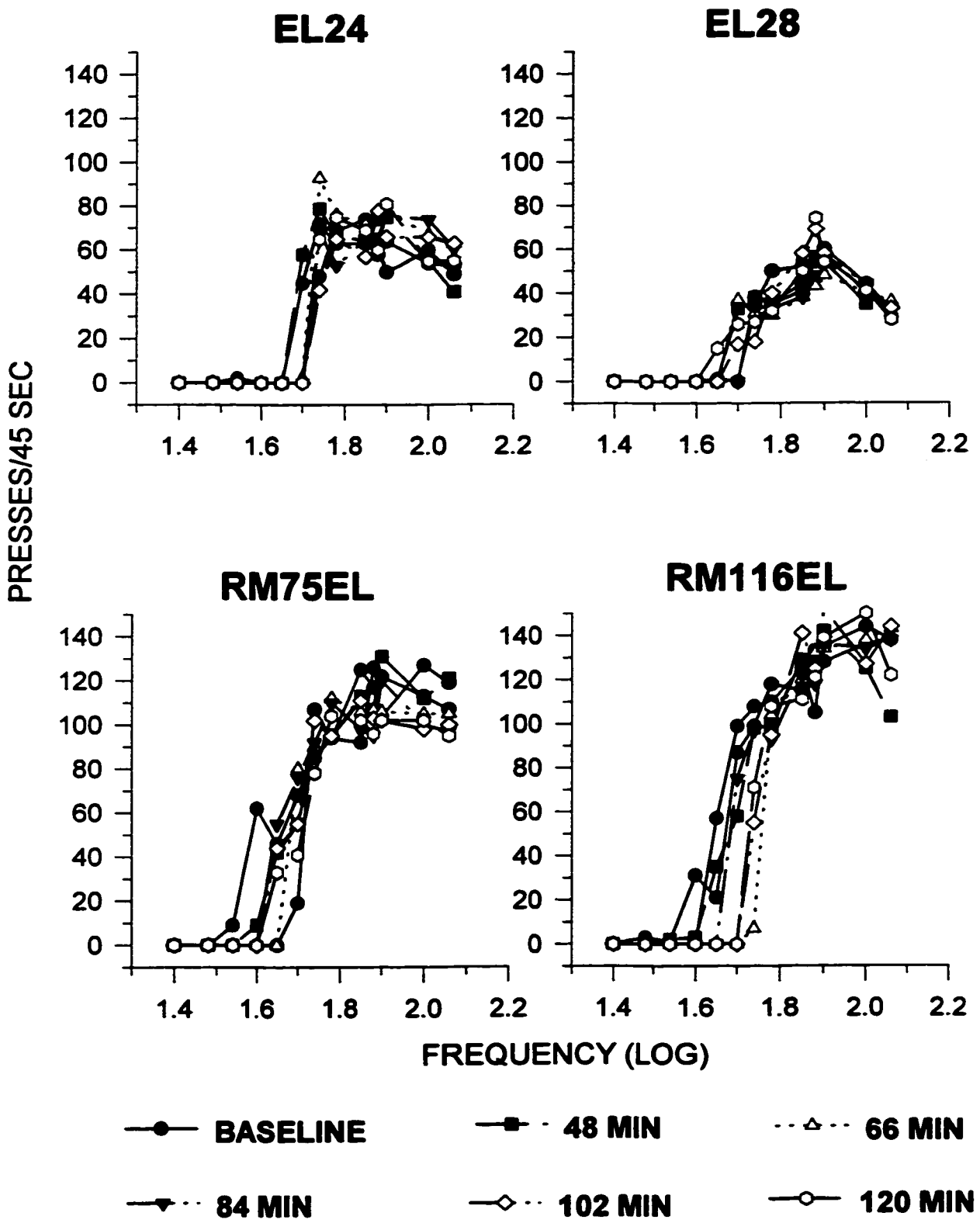
Figure 9 A, B, C. Rate of bar pressing as a function of the stimulation frequency (in log) obtained before, and at 48, 66, 84, 102 and 120 min after, injection of 160 µg/kg of SR-48692.

A

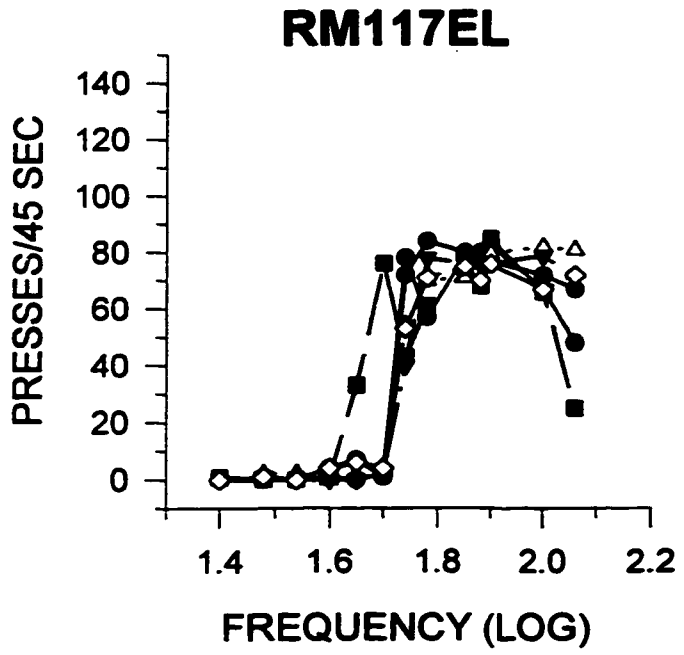

160 µg/kg SR-48692
EL2
EL7
EL9
EL15
PRESSES/45 SEC
FREQUENCY (LOG)
BASELINE
48 MIN
66 MIN
84 MIN
102 MIN
120 MIN

B

160 µg/kg SR-48692



C 160 µg/kg SR-48692

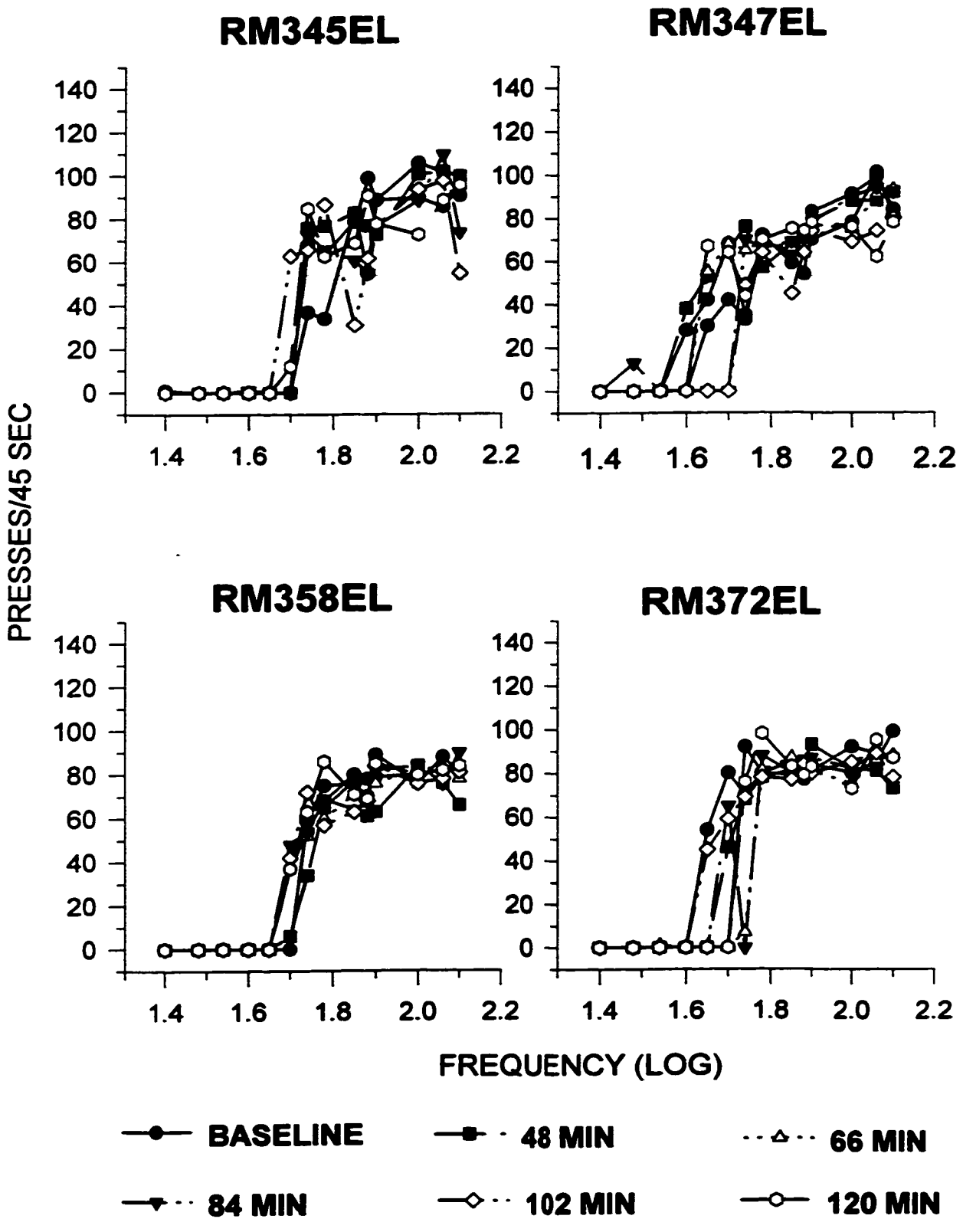


BASELINE 48 MIN 66 MIN

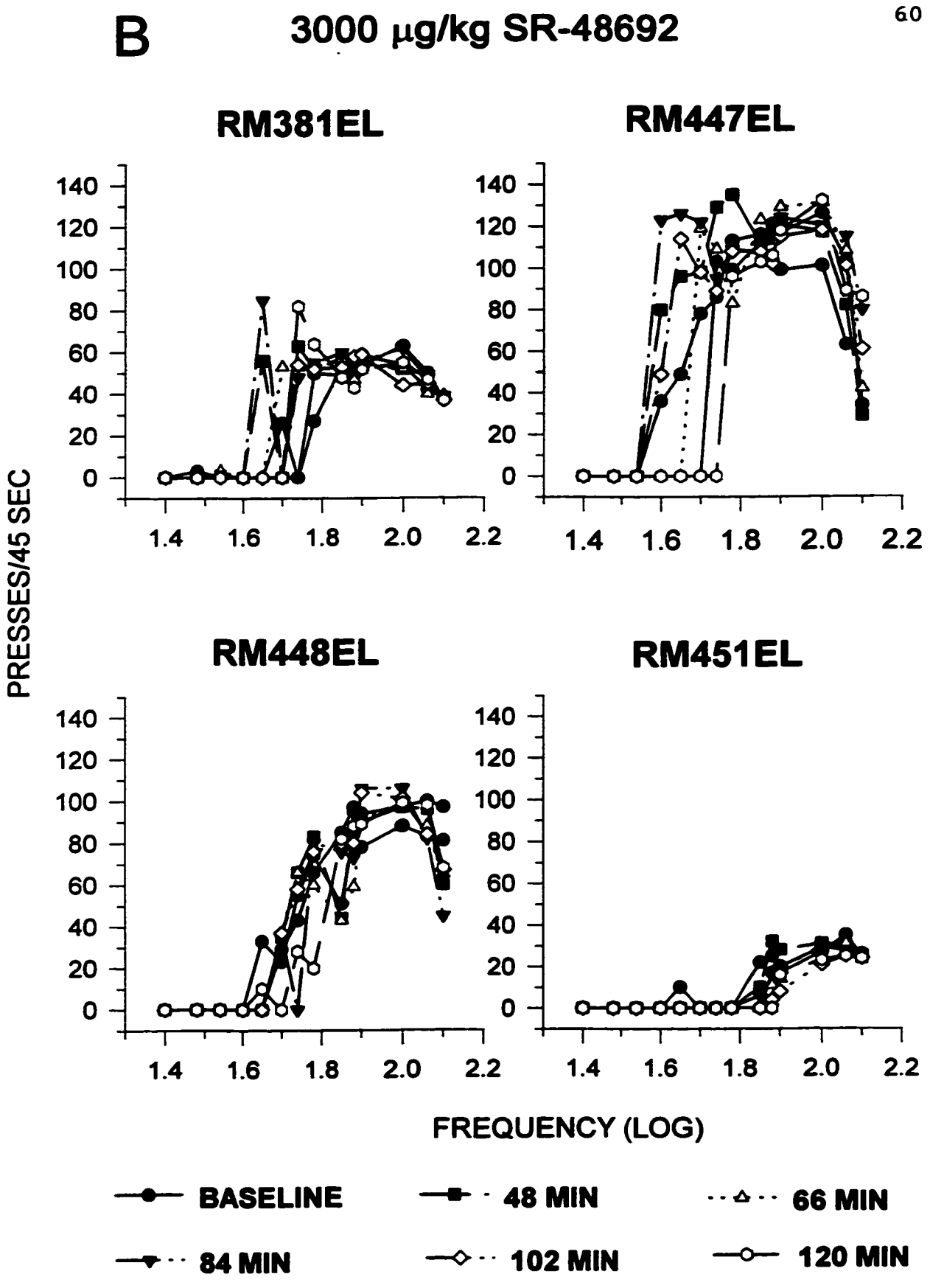
84 MIN 102 MIN 120 MIN

Figure 10 A, B, C. Rate of bar pressing as a function of the stimulation frequency (in log) obtained before, and at 48, 66, 84, 102 and 120 min after, the injection of 3000 μg/kg of SR-48692.

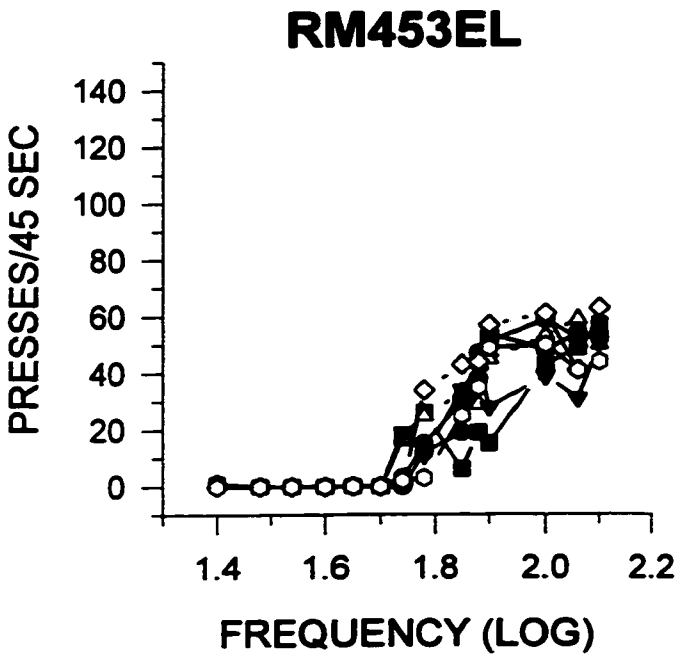
A


3000 µg/kg SR-49692
RM345EL
RM347EL
RM358EL
RM372EL
PRESSES/45 SEC
FREQUENCY (LOG)
BASELINE
48 MIN
66 MIN
84 MIN
102 MIN
120 MIN

B

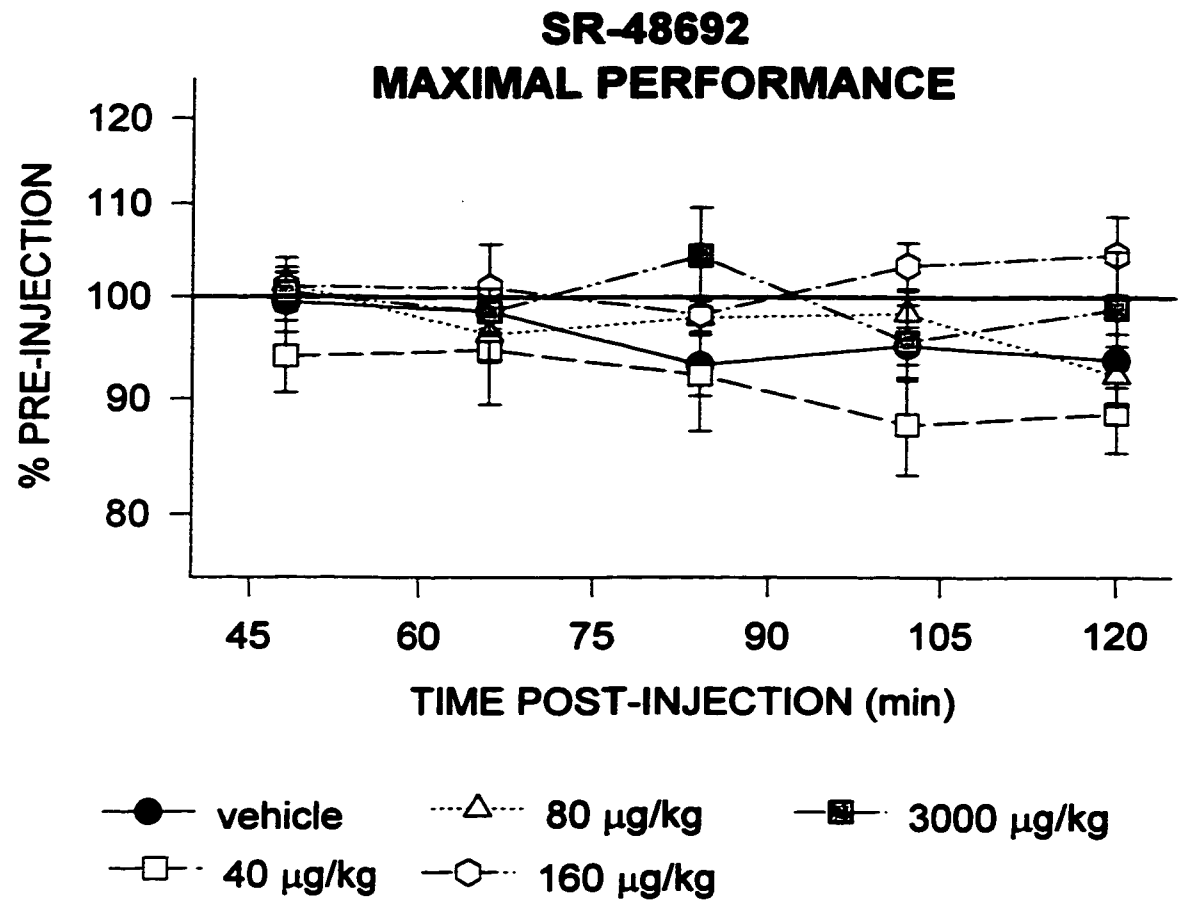

3000 µg/kg SR-48692
RM381EL
RM447EL
RM448EL
RM451EL
PRESSES/45 SEC
FREQUENCY (LOG)
BASELINE
48 MIN
66 MIN
84 MIN
102 MIN
120 MIN

C 3000 μg/kg SR-48692



BASELINE
48 MIN
66 MIN
84 MIN
102 MIN
120 MIN

Figure 11. Mean (±sem) changes in maximal rates of responding (top panel) and in reward threshold (bottom panel, M50 index) as a function of time after injection of vehicle, 40, 80, 160, and 3000 μg/kg of SR-48692. The data are expressed as percentages of pre-injection value.


SR-48692
MAXIMAL PERFORMANCE
% PRE-INJECTION
120
110
100
90
80
45
60
75
90
105
120
TIME POST-INJECTION (min)
vehicle
80 µg/kg
3000 µg/kg
40 µg/kg
160 µg/kg

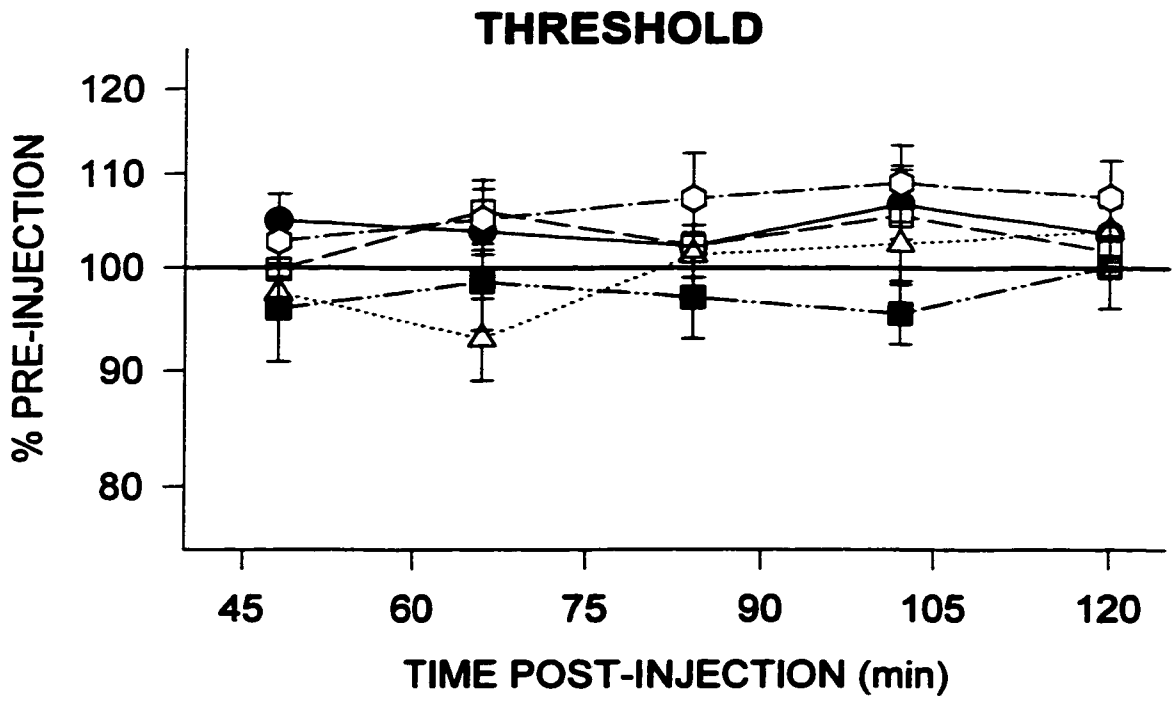
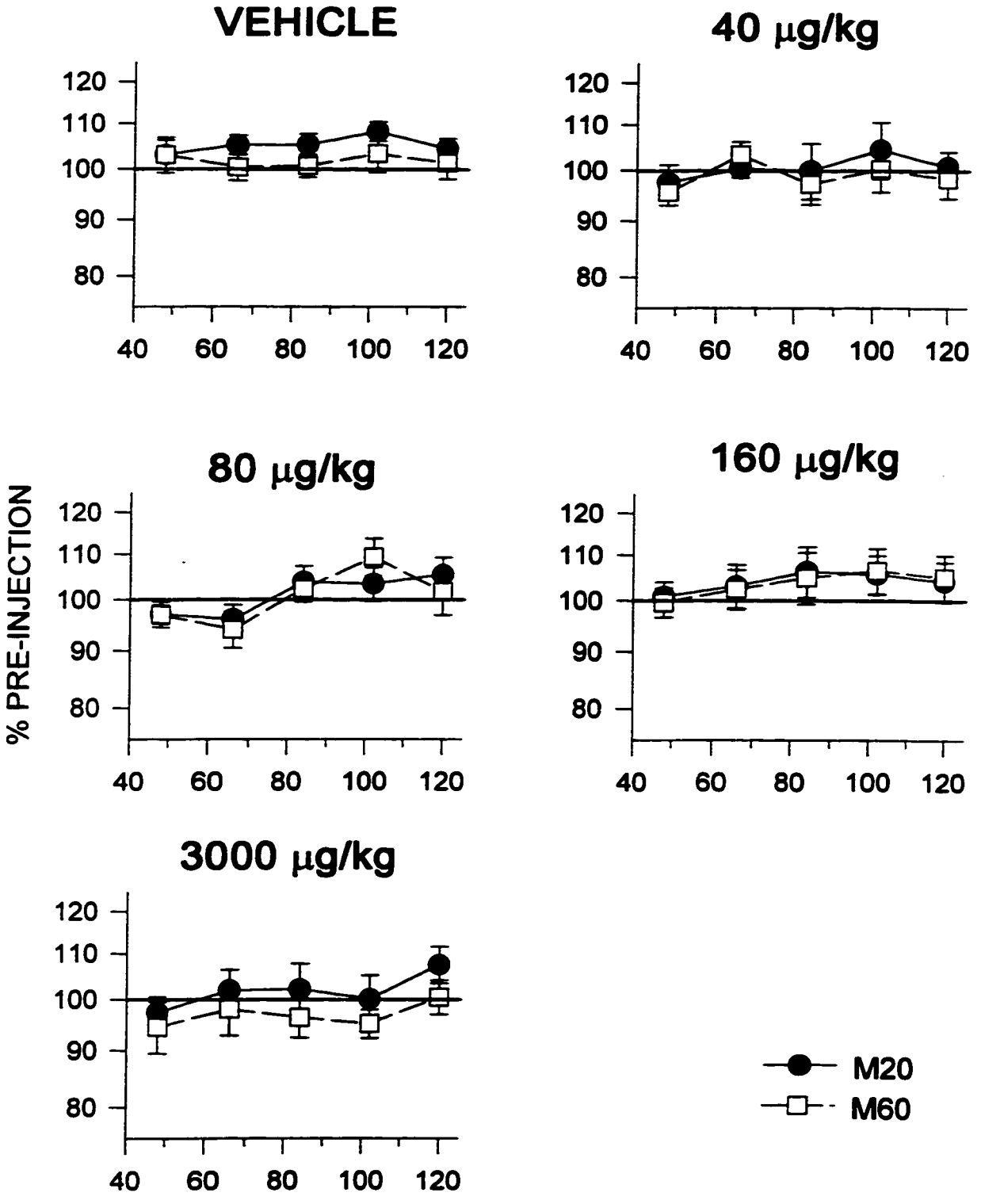

THRESHOLD
% PRE-INJECTION
120
110
100
90
80
45
60
75
90
105
120
TIME POST-INJECTION (min)

Figure 12. Comparison between the M20 and M60 measure of reward threshold as a function of time after injection of vehicle, 40, 80, 160, and 3000 ug/kg of SR-48692. The data (mean ±sem) are expressed as percentages of pre-injection value.

SR-48692
COMPARISON M20 VS M60



As previous analysis demonstrated that maximal rates of responding were not altered by SR-48692 and its vehicle, changes in reward threshold were expected to be dependent upon the level of the behavioural when the slope of the R-F had changes. The results of this analysis are presented in Fig. 12. As can be seen, estimates of the changes in reward threshold obtained following injection of each dose of SR-48692, and its vehicle, were not dependent upon the behavioural criterion. A statistical analysis (Paired T-tests, alpha = 0.083 [0.05 / 6, number of comparisons]) revealed no significant differences between the estimates obtained with M20 and M60, at each time for each treatment.

Comparison of the M50 and Theta 0 indices. To further test of the accuracy of the M50 index, M50 index was performed was compared it to the theta 0 index originally proposed by Miliaressis et al (1983). The theta 0 index corresponds to the highest stimulation frequency that fails to induce responding; it was inferred from the R-F function using a procedure previously described (Rompré and Wise, 1989). Miliaressis et al. (1986) have showed that the theta 0 is less sensitive (i.e. distorted) than the M50 index to variables that suppressed operant responding. Since the maximal rates of responding were not significantly suppressed following injections of each of the four doses of SR-48692, it was expected that estimates of reward threshold obtained with Theta 0 and with M50 would not differ.

Results of this analysis are presented in Fig. 13. As can be seen, estimates of the changes in reward threshold obtained following injection of each dose of SR-48692, and its vehicle, were again independent of the behavioural criterion. The statistical analysis (Paired T-tests, alpha = 0.083 [0.05 / 6, number of comparisons]) revealed no significant differences between estimates obtained with M20 and M60, at each time for each treatment.

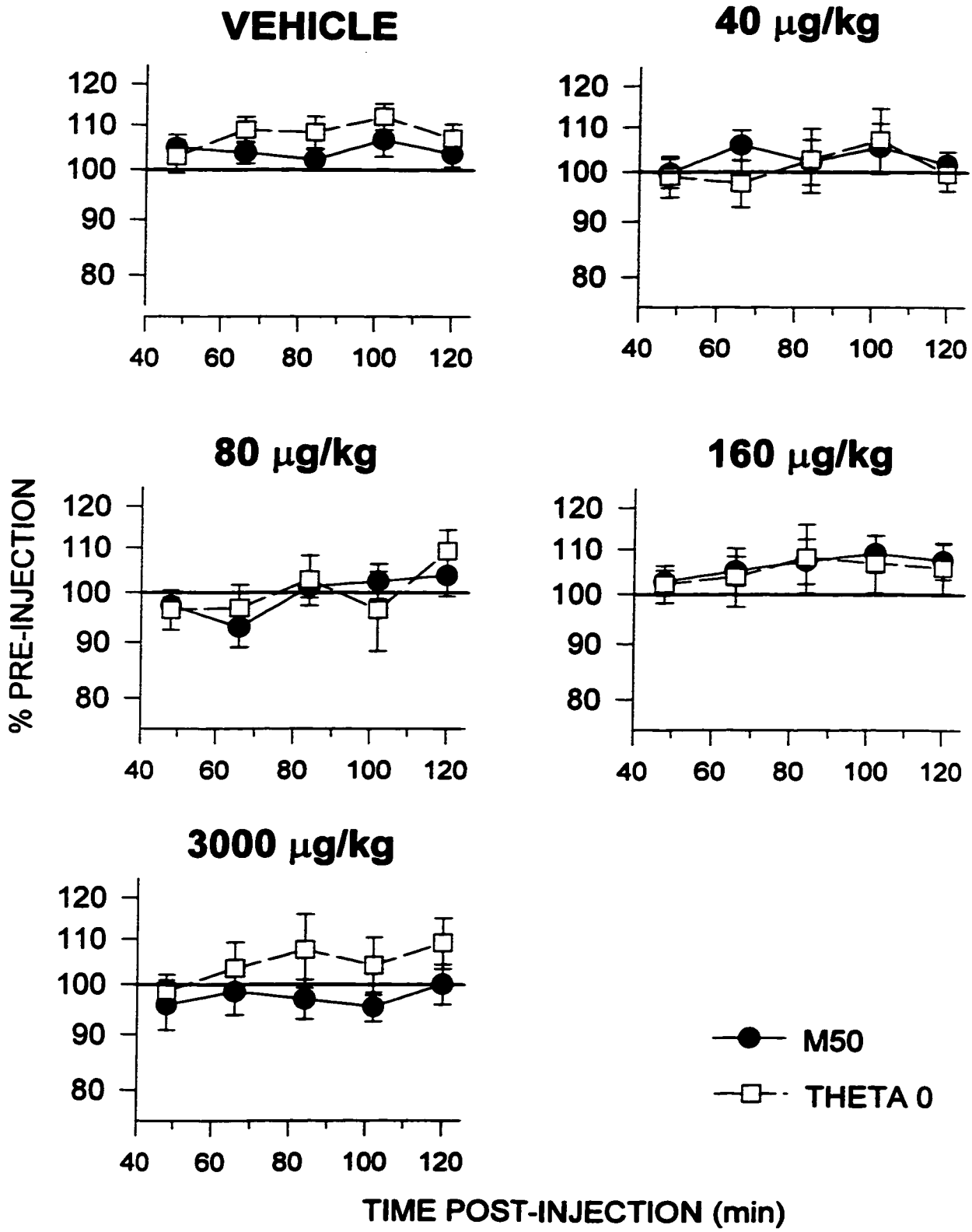
SR-142948a

Rate-Frequency Functions. Seven rats that were tested with SR-48692 were used to assess the effect of three doses of another selective neurotensin antagonist, the SR-142948a. Two rats had previously been injected with the vehicle, one rat with 80 μg/kg of SR-48692, and four rats with 3000 μg/kg.

The rate-frequency functions obtained before, and after, injection of each of the three doses of SR-142948a, and for each rat, are presented in Figs. 14 to 16. At the lowest dose (Fig. 14a and 14b, 40 μg/kg), SR-142948a had weak effects on the R-F functions; in effect, for most of the rats, maximal rates of responding changed little as the position of the functions on the x-axis. In the case of rat RM480EL, a small leftward shift that peaked at 102 min after the injection with no noticeable changes in maximal rates of responding was observed.

Figure 13. Comparison between the M50 and theta 0 measures of reward threshold as a function of time after injection of vehicle, 40, 80, 160, and 3000 μg/kg of SR-48692. The data (mean ±sem) are expressed as percentage of pre-injection value.

SR-48692
COMPARISON M50 VS THETA 0

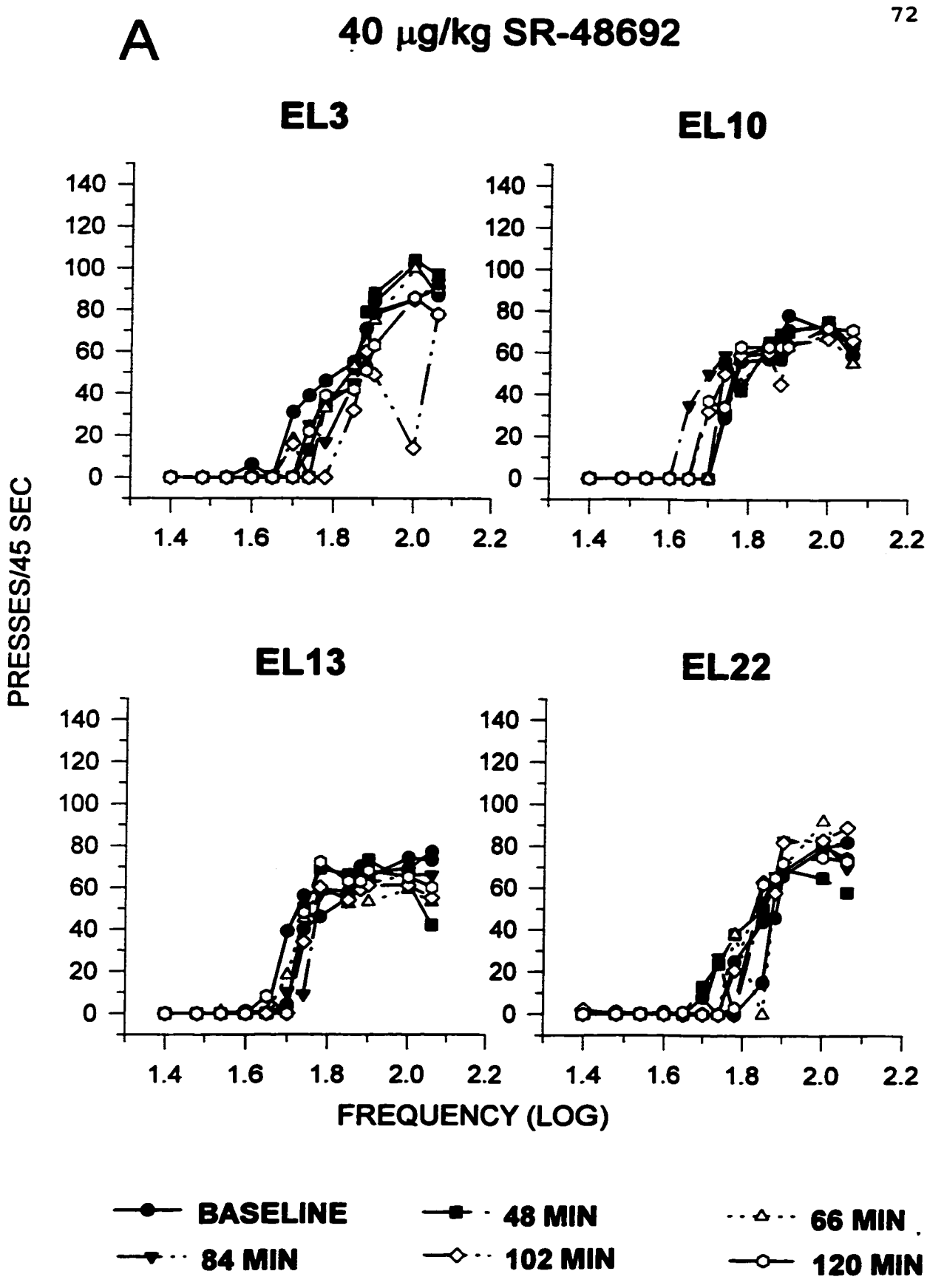


At 160 μg/kg, (Fig. 15a and 15b), SR142948a produced also weak effects on the R-F functions for most of the rats; one exception was rat RM447EL, which needed less stimulation to initiate responding resulting in a leftward shift of the R-F function. As seen with the previous dose, maximal rates of responding did not change.

At the highest dose (Fig. 16a and 16b, 640 μg/kg), SR-412948a was not more effective at altering the R-F functions. The exception was again rat RM447EL which needed much less stimulation to initiate responding; that resulted in a near 0.2 log unit leftward shift of the R-F function that peaked at 102 min post-injection. The maximal rates were not changed or slightly changed (RM480EL and RM481EL).

Maximal Rates and Reward Threshold. Figure 17 (top panel) shows the changes in maximal rates of responding (expressed in percentage pre-injection) as a function of time after the injection of 40, 160 and 640 μg/kg of SR-142948a. The solid horizontal lines representing the 95% confidence interval calculated from vehicle data collected in the previous experiment were used as an indication of the variability of the response following the injection. In general, SR-142948a had weak effect on maximal rates of responding. The largest changes occurred 120 min after the injection, a suppression and an increase at the lowest and the highest dose respectively.

Figure 14 A, B. Rates of bar pressing as a function of the stimulation frequency (in log) measured before, and 48, 66, 84, 102 and 120 min after, injection of 40 μg/kg of SR-142948a.


A
40 µg/kg SR-48692
EL3
EL10
EL13
EL22
PRESSES/45 SEC
FREQUENCY (LOG)
BASELINE
48 MIN
66 MIN
84 MIN
102 MIN
120 MIN

B

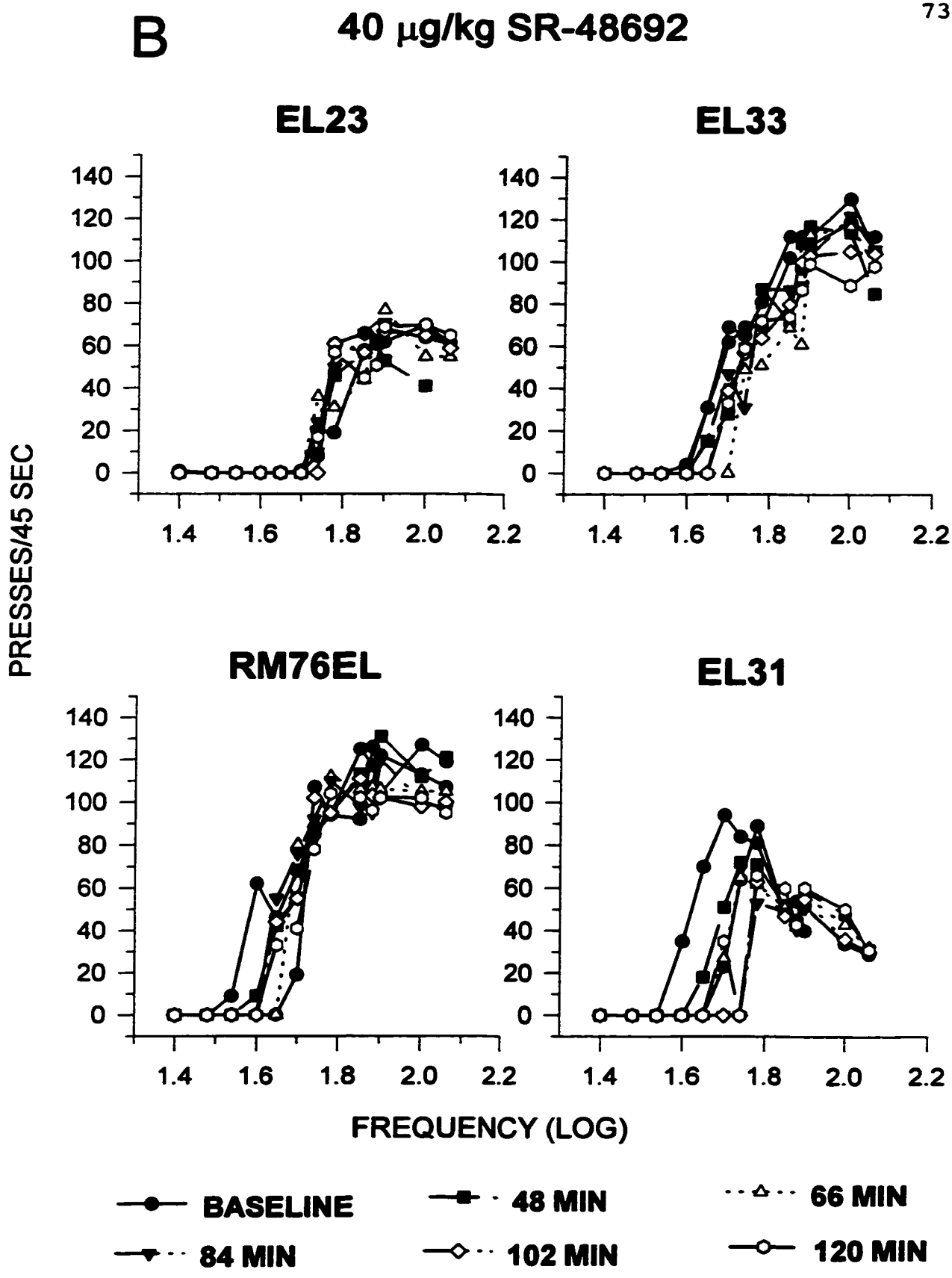
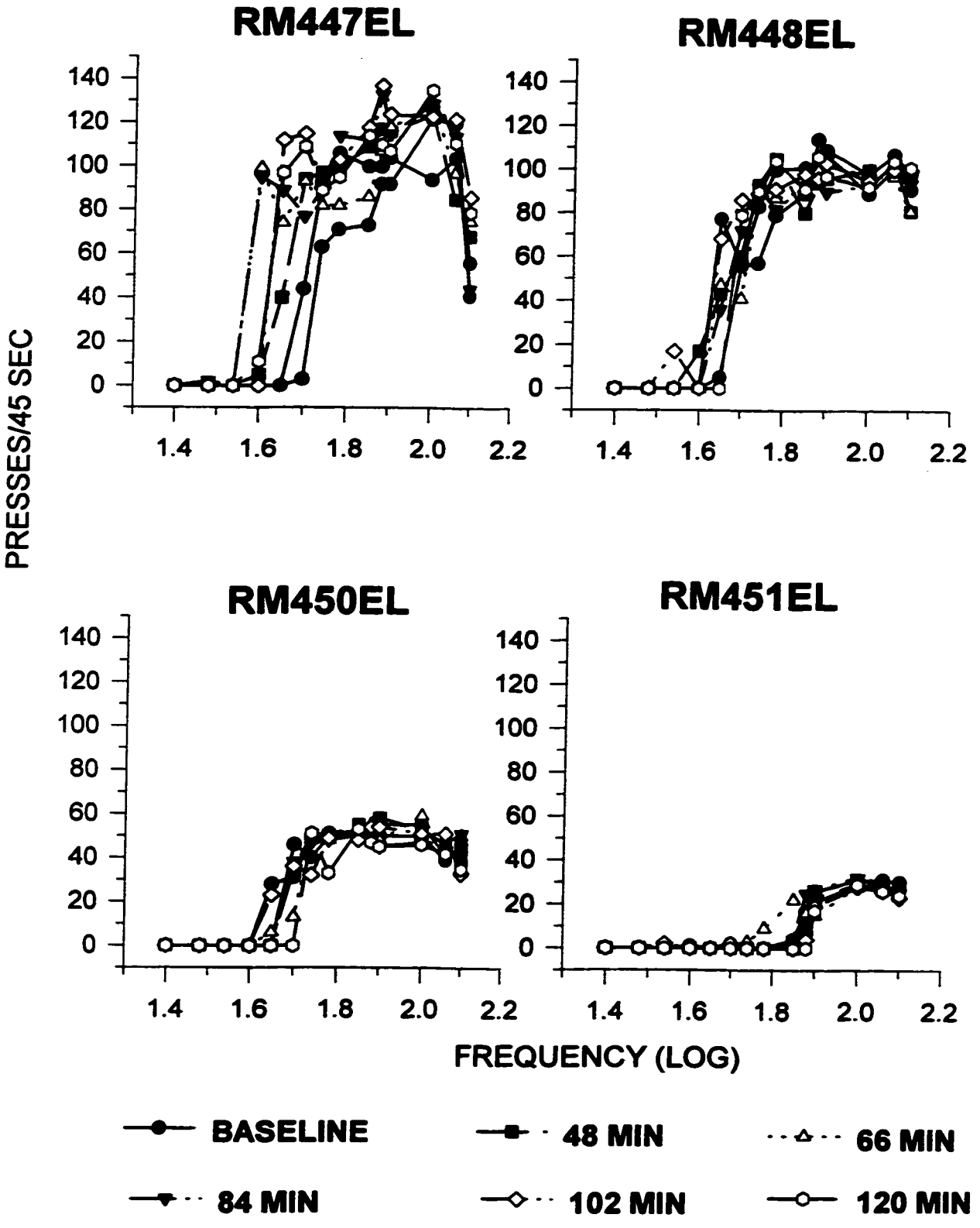

40 µg/kg SR-48692
EL23
EL33
RM76EL
EL31
PRESSES/45 SEC
FREQUENCY (LOG)
BASELINE
48 MIN
66 MIN
84 MIN
102 MIN
120 MIN

Figure 15 A, B. Rates of bar pressing as a function of the stimulation frequency (in log) measured before, and at 48, 66, 84, 102 and 120 min after, injection of 160 µg/kg of SR-142948a.

A

160 µg/kg SR-142948a



B

160 µg/kg SR-142948a

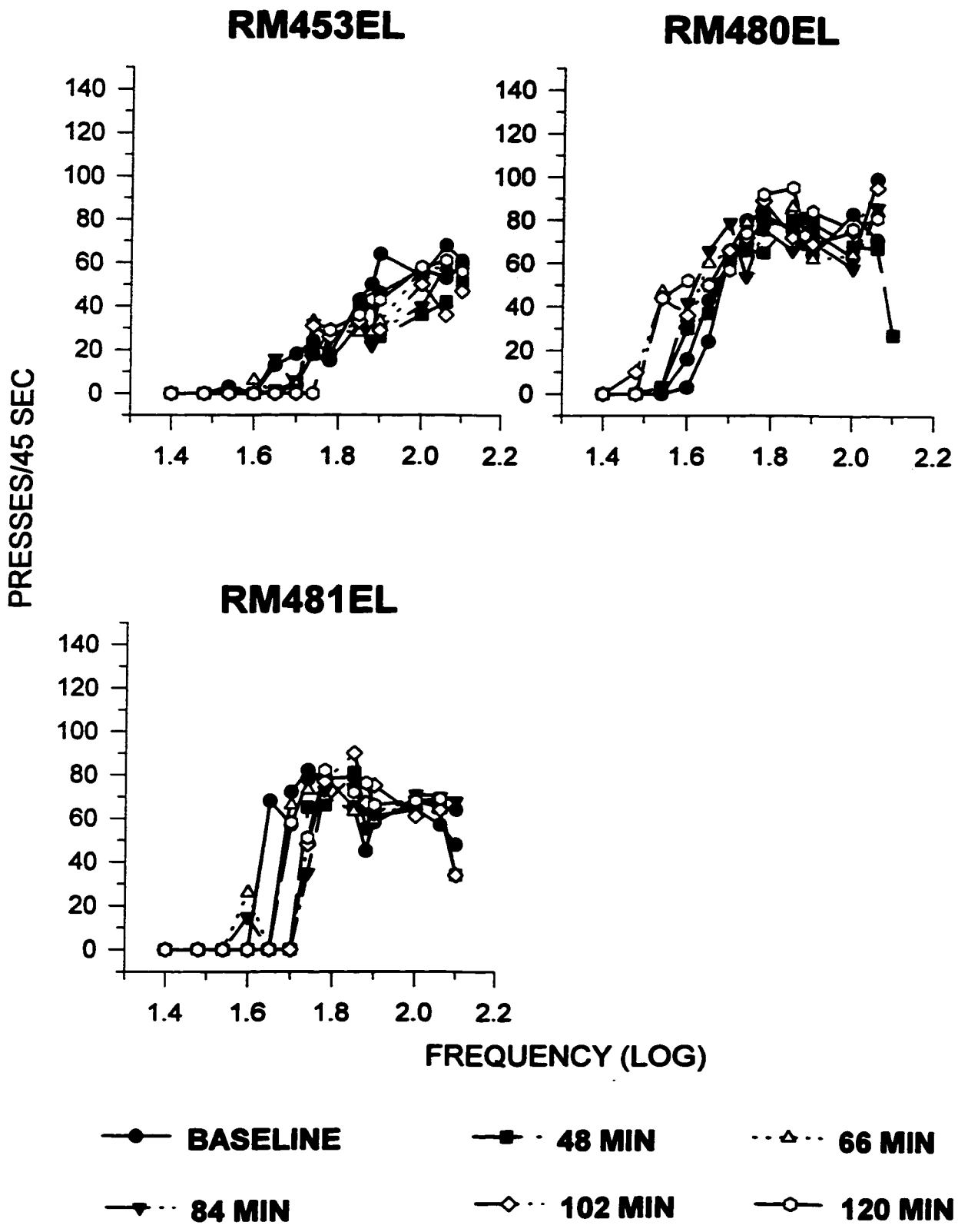
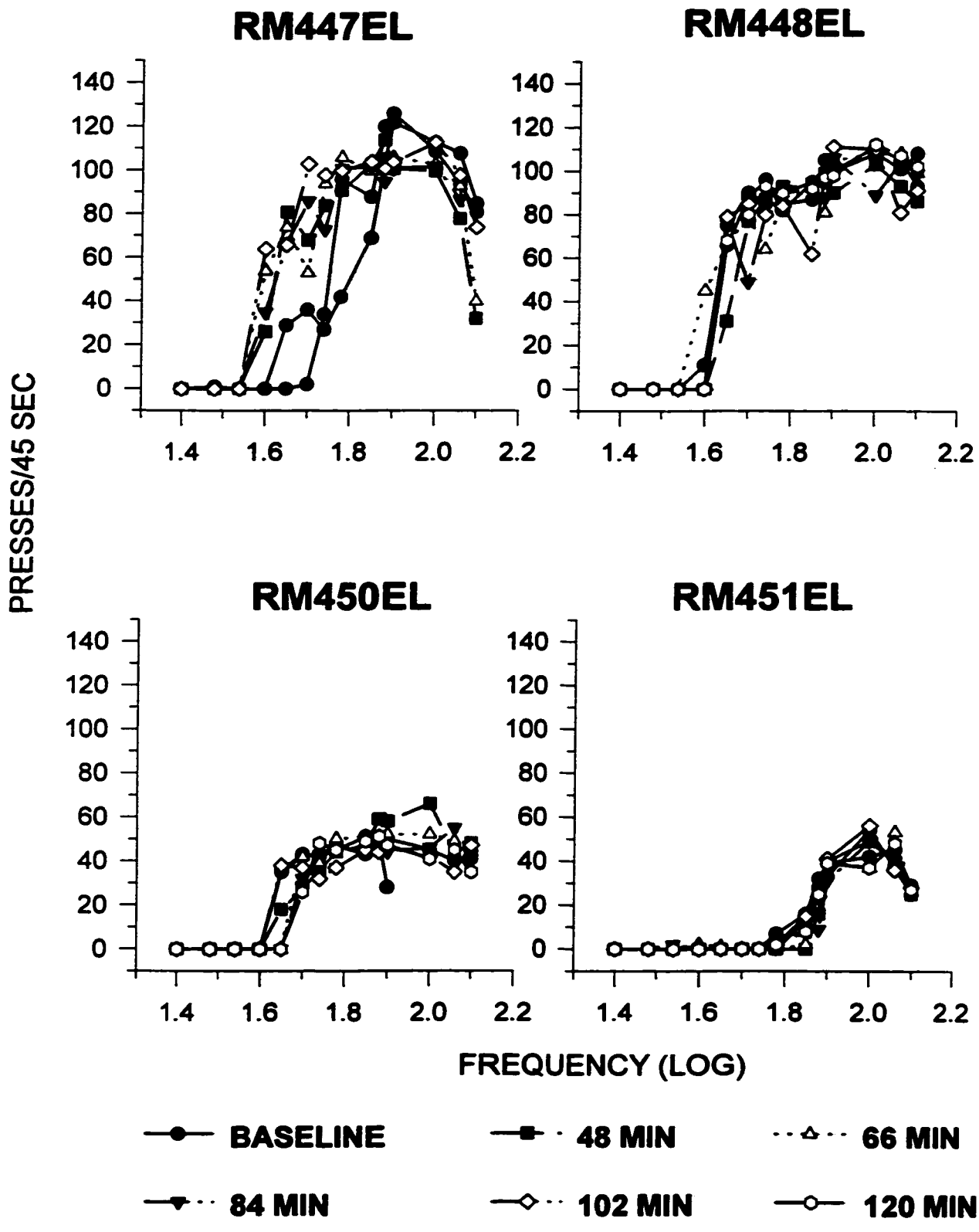


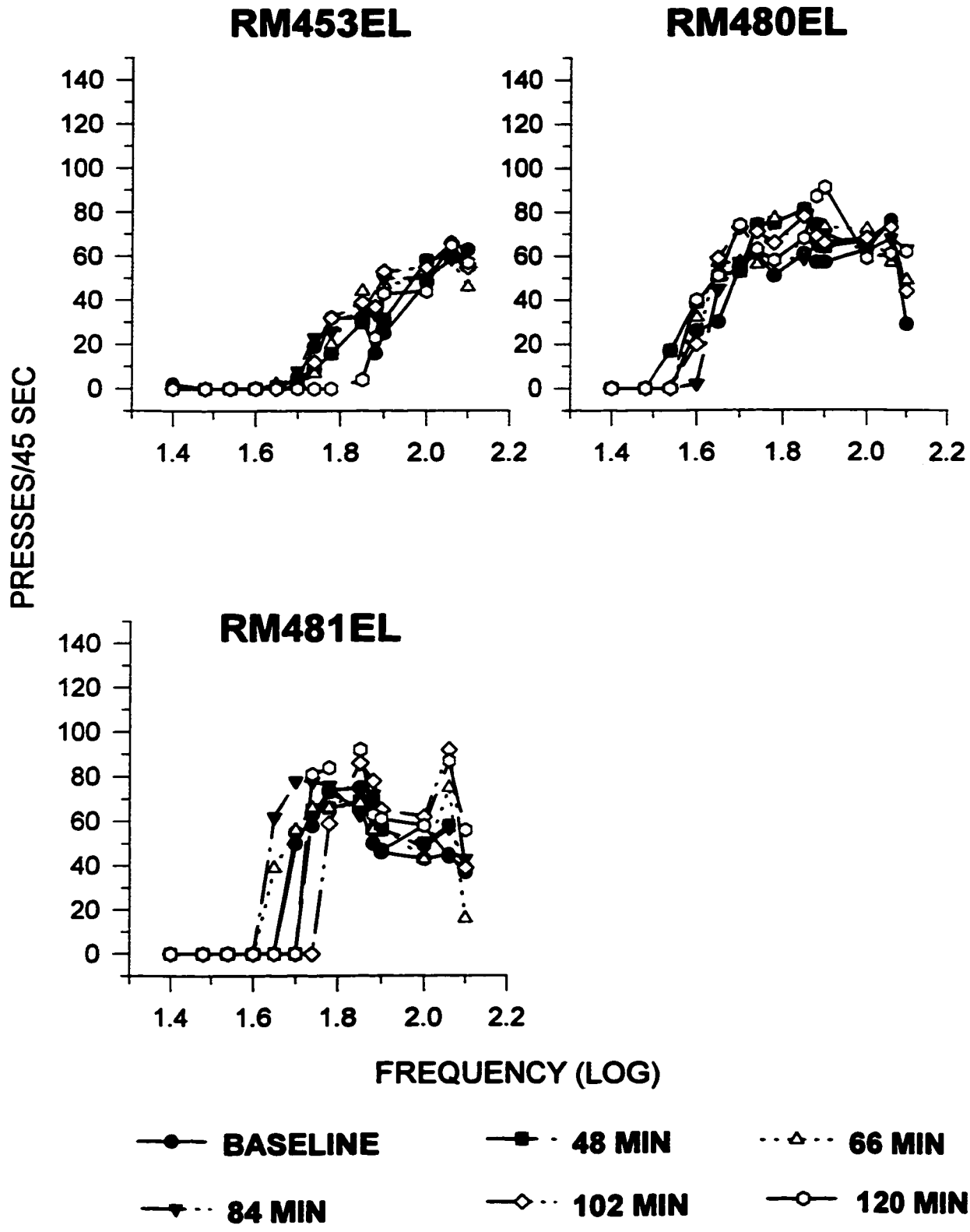
Figure 16 A, B. Rates of bar pressing as a function of the stimulation frequency (in log) measured before, and at 48, 66, 84, 102 and 120 min after, injection of 640 μg/kg of SR-142948a.

A

640 µg/kg SR-142948a



B 640 µg/kg SR-142948a

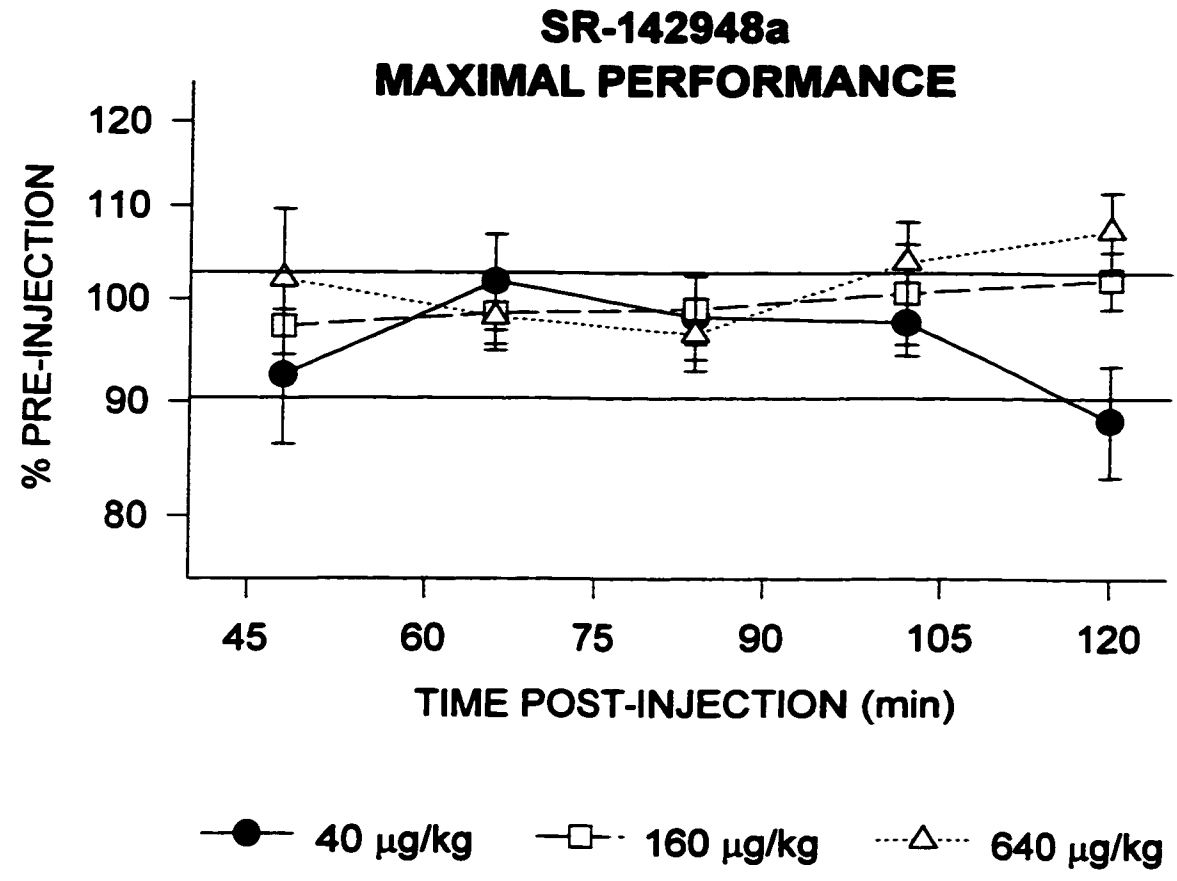


Changes in reward thresholds (expressed in percentage pre-injection) as a function of time after the injection of 40, 160 and 640 μg/kg of SR-142948a are shown in the bottom panel of Fig. 17. It can be seen that relative threshold changes measured after the injection of each of the three doses of the neurotensin antagonist were mostly within the 95% confidence interval, except at the highest dose level at 48 and 120 min after the injection and at the lowest dose level at 120 min.

Comparison of the M50 and Theta 0 indices. A test of the accuracy of the M50 index was performed by comparing it to the Theta 0 index originally proposed by Miliaressis et al (1983). The results are presented in Fig. 18. Paired T-tests revealed that the results obtained with theta 0 and M50 did not significantly differ at any of the doses and of the 5 time periods post-injection ($P > 0.05 / 6$).

Slope of the R-F functions. In order to determine whether the administration of SR-142948a altered the slope of the R-F function, we performed a statistical comparison of the changes in reward threshold measured with the M20 and M60 was performed (Fig. 19). As can be seen, the estimates of the changes in reward threshold obtained following injection of each of the three doses of the neurotensin antagonist were independent of the behavioural criterion. Statistical analysis (Paired T-tests, alpha = 0.083 [0.05 / 6, number of comparisons]) revealed no significant difference between the estimates obtained with M20 and M60, for each treatment.

Figure 17. Mean (±sem) changes in reward threshold and maximal rates of responding as a function of time after injection of 40, 160 and 640 μg/kg of SR-142948a. The two horizontal lines represent the lower and the higher level of the 95% confidence interval calculated from the group that received a vehicle injection.


SR-142948a
MAXIMAL PERFORMANCE
% PRE-INJECTION
120
110
100
90
80
45
60
75
90
105
120
TIME POST-INJECTION (min)
40 µg/kg
160 µg/kg
640 µg/kg

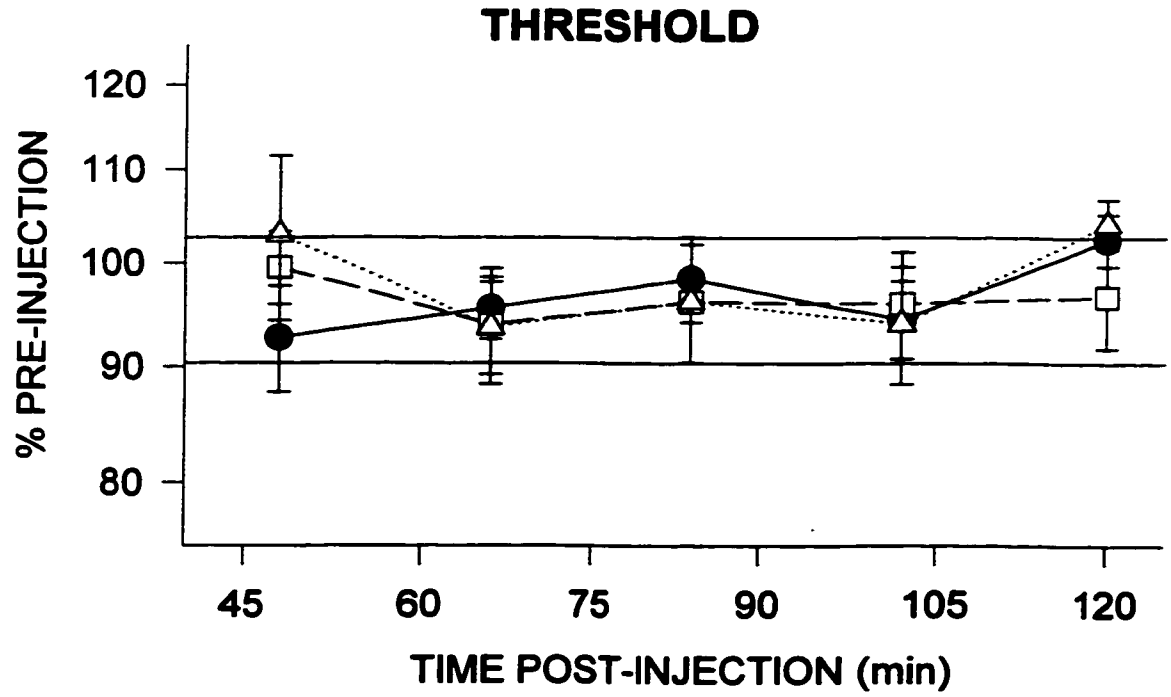

THRESHOLD
% PRE-INJECTION
120
110
100
90
80
45
60
75
90
105
120
TIME POST-INJECTION (min)

Figure 18. Comparison between the M20 and M60 measures of reward threshold as a function of time after injection of vehicle, 40, 160, and 640 μg/kg of SR-142948a. Data (mean ±sem) are expressed as percentage of pre-injection value.

SR-142948a

COMPARISON M20 VS M60

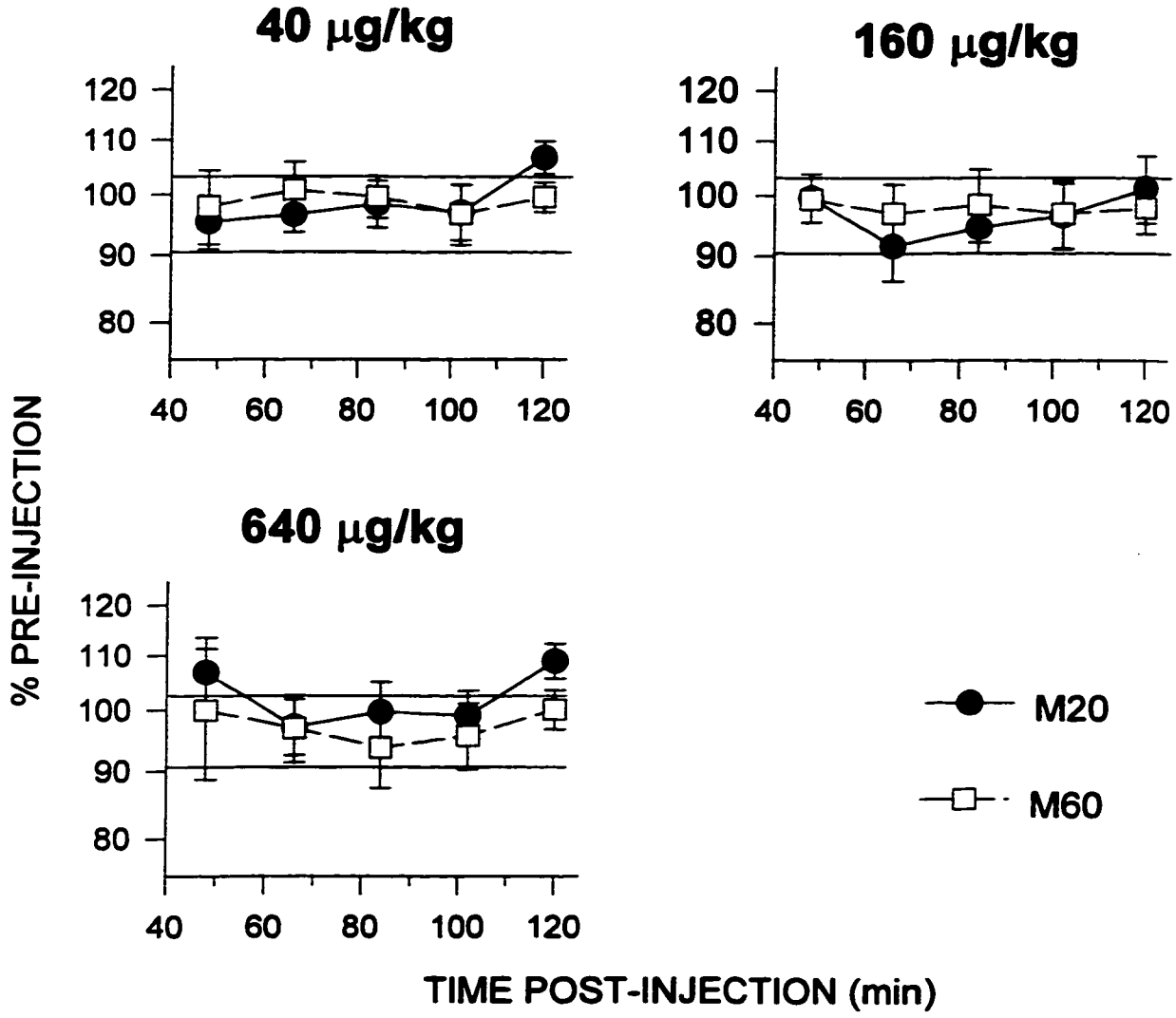
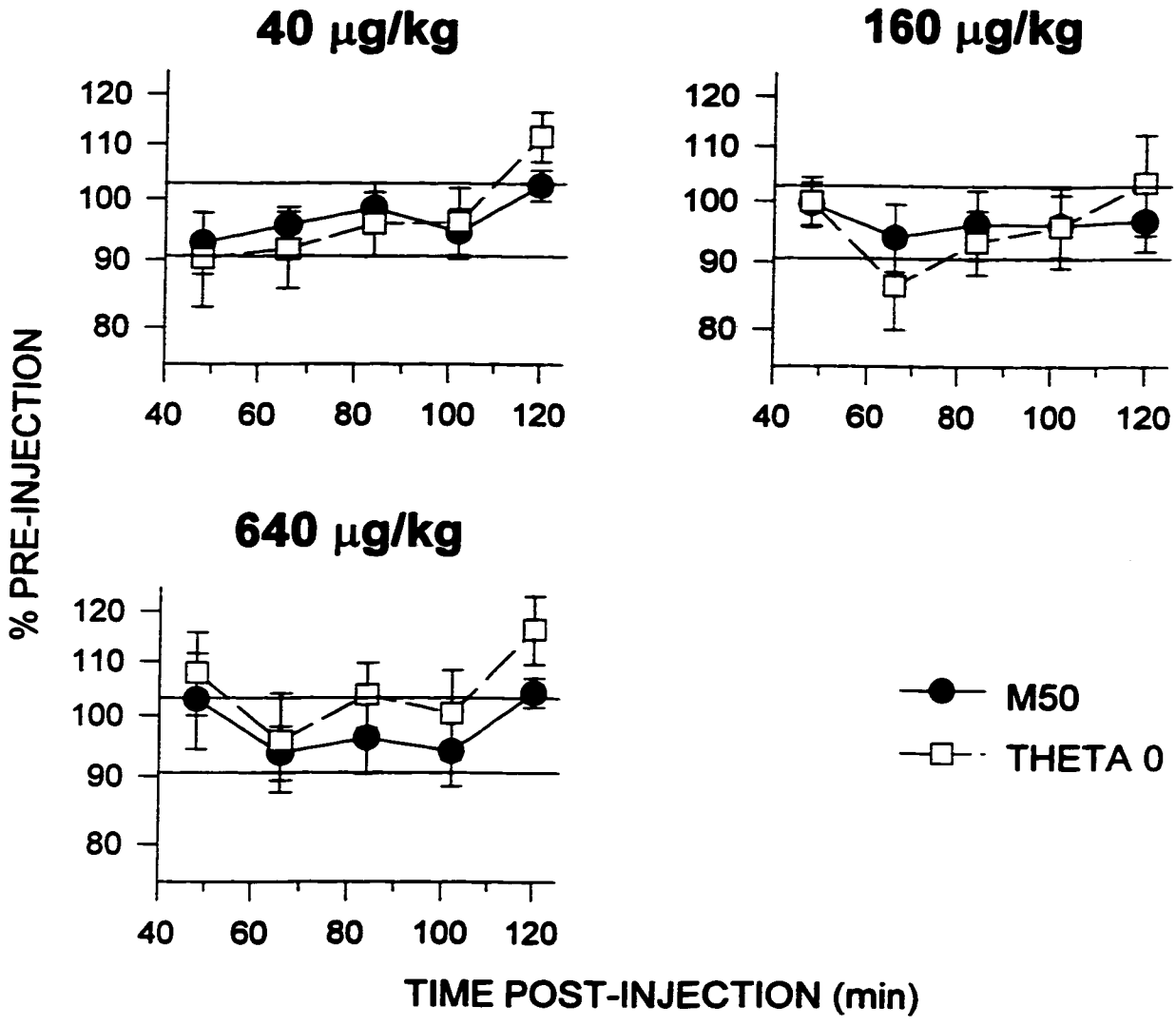


Figure 19. Comparison between the M50 and Theta 0 measures of reward threshold as a function of time after injection of vehicle, 40, 160, and 640 μg/kg of SR-142948a. Data (mean ±sem) are expressed as percentage of pre-injection value.

SR-142948a

COMPARISON M50 VS THETA 0



Discussion

This study was aimed at determining whether NT is an important component of the reward-relevant circuitry. To reach this objective, the effects of two selective NT antagonists, SR-48692 and SR-142948a, were tested on several parameters of responding for rewarding electrical stimulation of the medial mesencephalon.

Responding for BSR. Forty-five out of the 67 rats initially prepared for the experiment were successfully trained to respond for BSR. Histological analyses of the brains of the 47 rats that self-administered the stimulation revealed the stimulation sites were located near, or within, the ventral central gray, between anterior-posterior levels corresponding to the rostral and caudal borders of the dorsal raphe nucleus. The location of positive BSR sites observed in the present study is consistent with previous mapping results revealing the existence of reward-relevant neurons within the medial mesencephalon, between the most caudal part of the dorsal raphe nucleus and the ventral tegmental area. (Rompré and Milliaressis, 1985; Rompré and Boye, 1989; Boye and Rompré, 1996).

Results with SR-48692. Analysis of the data obtained after injection of the four doses of SR-48692 revealed that this selective NT antagonist did not alter any of the parameters of BSR measured in this study.

First, SR-48692 injection failed to alter asymptotic rates of responding at any of the doses tested. Because this parameter of operant responding reflects, at least in part, the capacity of the animal to produce the operant response, it can be concluded that blockade of SR-48692-sensitive NT receptors does not alter performance variables.

Second, it was inferred from the lack of differences between reward thresholds measured with a low (M20) and a high (M60) behavioral criterion that SR-48692 injection did not alter the slope of the R-F function as. This observation provides additional support for the hypothesis that blockade of SR-48692-sensitive NT receptors does not alter performance variables. In effect, in the model proposed by Edmonds and Gallistel (1974), later validated for the operant box by Miliaressis et al (1986), a decrease in performance results in a decrease in asymptotic responses and in a suppression of the slope of the R-F function. The lack of effect on the slope of the R-F function also suggests that the rate of increase in the rewarding effectiveness of the stimulation as a function of the stimulation frequency is not altered by blockade of SR-48692-sensitive NT receptors.

Third, the rewarding effectiveness of the stimulation was not altered by any of the doses of SR-48692 tested as assessed using the M50 index. Similar results were obtained using the theta 0 index. Such a lack of effect on reward threshold suggests that blockade of SR-48692-sensitive NT receptors does not

alter the reward signal induced by electrical stimulation of the medial mesencephalon.

It is noteworthy, however, that for some rats, results were divergent with the average. In the case of EL31, for instance, a rightward shift of the R-F function was observed 120 min after the injection of 40 μg/kg of SR-48692, an effect not seen at higher doses. Interestingly, the stimulation site for this rat was not at odds with these of the other rats, excluding activation of a different populations of reward-relevant neurons within the medial mesencephalon. In the case of EL7, a leftward shift of the R-F function was observed 84 min after injection of 160 μg/kg of SR-48692, suggesting, at this dose and at this time, a potentiation of the rewarding effectiveness of the stimulation. The stimulation site for this rat was located more anteriorly to those of the other rats, in the anterior and lateral part of the dorsal raphe nucleus. RM480EL showed a suppression of the rate of responding at 48 min after a injection of 80 μg/kg of SR-48692, an effect not seen at other doses or at other times in any other rats. Again it is unlikely that anatomical variables can account for the above observations.

One rat, RM447EL, had a stimulation site that differed from all the other rats tested in this study; it was located about 1.3 mm more anteriorly, near the Edinger-Westphal nucleus. Interestingly, this rat showed sensitivity to SR-48692. At the highest dose, the R-F curve was shifted to the left at 48, 84 and 102 min after the injection, an effect that was also observed, with the same rat, with the

other NT antagonist tested (see later). This anecdotal result tends to suggest that location of the stimulation site, hence the subpopulation of reward-relevant neurons activated, is a relevant variable.

Based on the grouped data, we can conclude that operant responding to electrical stimulation of the medial mesencephalon is insensitive to blockade of NT receptors by SR-48692-sensitive NT receptors. Several factors can account for this lack of effect and deserve to be discussed.

The negative results are unlikely to be due to the range of doses tested, or to a poor brain penetration of the drug. In effect, several studies have shown that, at a similar range of doses, SR-48692 was effective at blocking the central effects of NT. At 40-160 µg/kg, for instance, SR-48692 blocked circling behavior induced by ventral mesencephalic injections of NT, and DA release induced by striatal injections of NT (Gully and al. 1993; Steinberg and al. 1994). At higher doses (1 m/kg), SR-48692 blocked DA agonists-induced striatal Fos activation (Alonso and al. 1997). In addition, a previous experiment performed in the laboratory used in this series of experiments, Perron and Rompré (1997) showed that SR-48692 prevents the development of sensitization to the locomotor activating effect of amphetamine; this finding demonstrates that the procedure applied to prepare the drug, and the doses used in the present study (80 and 160 µg/kg), could not account for the negative results.

One hypothesis that may account for the negative results recorded here is that the opposite effects of SR-48692 at the DA terminal and DA cell body levels may have canceled each other out. This hypothesis could be postulated on the basis of the previous reports that showed that the activation of NT receptors in the ventral mesencephalon and in the ventral striatum respectively potentiates and inhibits operant responding for reward (Nemeroff et al., 1982; Rompré et al., 1993). It would be unlikely, however, that the effects of SR-48692 at both sites would perfectly cancel out at every dose tested.

The negative results can also be explained by a specificity of SR-48692 to a sub-type of NT receptors not involved in the NT-induced potentiation of BSR. This hypothesis is supported by previous studies showing that SR-48692 blocked some, but not all, central effects of NT. SR-48692 was found ineffective, over a large range of doses, at blocking NT-induced hypothermia and analgesia (Dubuc et al., 1994). Moreover, pretreatment with SR-48692 failed to prevent ventral striatal DA release induced by mesencephalic NT microinjections (Steinberg et al., 1994) and NT-induced increase in DA cell firing (Nalivaiko et al., 1998).

The hypothesis of the specificity of SR-48692 for a sub-type of NT receptor was indirectly tested in the present study with the new NT antagonist SR-142948a. In addition to blocking all the physiological effects of NT that were blocked by SR-48692, SR-142948a is also effective at blocking NT-induced hypothermia and analgesia (Gully et al., 1997). The results obtained showed that

all parameters of BSR measured were insensitive to a range of behaviorally-relevant doses of SR-142948a. This strongly suggests that sub-types of NT receptors blocked by the two NT antagonists are not important components of the reward-relevant circuitry.

Taken together, these findings tend to suggest that NT itself is not an important component of the reward-relevant pathway activated by electrical stimulation of the medial mesencephalon. This hypothesis is, at first glance, difficult to reconcile with previous reports that ventral mesencephalic microinjections of NT potentiate BSR, and are, by themselves, sufficient to produce rewarding effects (Rompre et al., 1993; Glimcher et al., 1982,1984, 1987). It could be that NT acts as a modulator of the reward-relevant pathway but that the modulatory mechanism is not activated during reward induced by electrical stimulation of the medial mesencephalon; this implies that the reward-relevant pathway is not under tonic control of endogenous NT. It is important to keep in mind, however, that the NT antagonists produced consistent alterations in reward in rat RM447EL. In this rat, leftward curve-shifts were observed following treatment with the highest dose of SR-48692 and following treatment with the two highest doses of SR-142948a. Interestingly, the stimulation site of this rat was more anterior than the stimulation sites of all the other rats tested. This suggests that the modulatoray action of NT can effectively be activated during BSR. It would be interesting to investigate the effect of the NT

antagonists on BSR again but with electrode placements located more anteriorly within the medial mesencephalon.

The hypothesis that NT is indeed an important component of the reward-relevant pathway, acting through a sub-type of receptors insensitive to SR48692 and SR-142948a received support from recent findings reported by Gully et al., (1997). In effect, they reported that SR-142948a, just like SR-48692, fails to block the increase in nucleus accumbens DA release produced by mesencephalic NT microinjections. Future studies may test the ability of SR-142948a to prevent the potentiation of BSR produced by mesencephalic NT microinjections; moreover, identification of the NT receptors involved in this potentiation effect of NT and the development of a new NT antagonist that will interact with this hypothetical receptor would greatly contribute to the clarification of this issue.

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Appendix 1

Table 1

Related t-test for asymptote: SR-142948a

Doses	df	t	p
40ug/kg			
48 min	6	.94	.39
66 min	6	-.45	.67
84 min	6	-.1	.92
102 min	6	.19	.85
120 min	6	2.36	.06
160ug/kg			
48 min	6	.61	.57
66 min	6	.22	.83
84 min	6	.16	.88
102 min	6	-.48	.65
120 min	6	.84	.43
640ug/kg			
48 min	6	.74	.49
66 min	6	.99	.36
84 min	6	1.24	.26
102 min	6	-.59	.57
120 min	6	-1.8	.13

Table 2

Related t-test for M50: SR-142948a

Doses	df	t	p
40ug/kg			
48 min	6	1.63	.16
66 min	6	1.52	.18
84 min	6	.74	.49
102 min	6	1.71	.14
120 min	6	-.68	.52
160ug/kg			
48 min	6	.32	.76
66 min	6	1.28	.25
84 min	6	.82	.44
102 min	6	.92	.39
120 min	6	.71	.51
640ug/kg			
48 min	6	-.79	.46
66 min	6	1.50	.18
84 min	6	.82	.44
102 min	6	1.13	.30
120 min	6	-1.69	.15