Title
Localized Inactivation of Neuronal Activity with Bupivacaine Elevates Brain Reward Thresholds in Rostral but not Caudal Insular Cortex /

Permalink
https://escholarship.org/uc/item/8c7791g7

Author
Gourvitz, Allison

Publication Date
2014

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA, SAN DIEGO

Localized Inactivation of Neuronal Activity with Bupivacaine Elevates Brain
Reward Thresholds in Rostral but not Caudal Insular Cortex

A thesis submitted in partial satisfaction of the requirements for the degree
Master of Science

in

Biology

by

Allison Gourvitz

Committee in Charge:

Professor Gerhard Schulteis, Chair
Professor Stephanie Mel
Professor Yunde Zhao

2014

The thesis of Allison Gourvitz is approved, and it is acceptable in quality and form
for publication on microfilm and electronically:

_____

_____

_____

Chair

University of California, San Diego

2014

Table of Contents

List of Figures and Tables

List of Abbreviations

RI, Rostral Insula

CI, Caudal Insula

VEH, Vehicle

BUP, Bupivacaine

AMPH, Amphetamine

aCSF, artificial cerebral-spinal fluid

ICSS, Intracranial self-stimulation

IC, Intracranially

SC, Subcutaneously

Acknowledgements

In addition to Professor Schulteis and the members of my committee, I would like to acknowledge the members of the Schulteis Lab Group who made completion of this thesis possible. Specifically: Clay Archer, David Zhang, Navarre Gutierrez-Reed, and Colleen Lonergan.

ABSTRACT OF THESIS

Localized Inactivation of Neuronal Activity with Bupivacaine Elevates Brain
Reward Thresholds in Rostral but not Caudal Insular Cortex

by

Allison Gourvitz

Master of Science in Biology

University of California, San Diego, 2014

Professor Gerhard Schulteis, Chair

Prior research has implicated interoceptive processing in the insular cortex
as a critical component in drug craving. The goal of this thesis was to elucidate
the role the insular cortex plays in the experience of the direct rewarding effects
of drugs, as measured by brain stimulation. Neuronal activity was inhibited by
infusion of the local anesthetic bupivacaine into the rostral insular cortex (RI) or
the caudal insular cortex (CI) prior to testing rats in an intracranial self-stimulation

(ICSS) paradigm to measure brain reward current intensity thresholds. Rats received either an injection of d-amphetamine (0.5 mg/kg) or saline vehicle 5 min after bupivacaine infusions. Inactivation of the RI but not CI elevated brain reward thresholds in amphetamine-naïve rats, suggesting the rostral insula with its interconnections with reward circuitry may modulate the experience of reward from brain stimulation. Inactivation of the RI or CI with infusions volumes that were inactive in amphetamine-naive rats did not alter the reward-enhancing effects of 0.5 mg/kg d-amphetamine, suggesting that this dose of d-amphetamine produces its reward-enhancing effects independent of its interoceptive effects which are integrated by the insula.

1   Introduction

Substance addiction is a major health problem in the United States that has detrimental impacts on individuals, families, and society as a whole. In 2011 the Center for Disease Control and Prevention's National Vital Statistics Report indicated that the number of drug-induced deaths in the United States reached 40,239 (Hoyert, 2012). Abuse of prescription and illegal drugs, excessive alcohol consumption, and smoking costs the United States more than 467 billion dollars per year (Califano 2009). Most of this money is allocated to dealing with the consequences of substance abuse and addiction that commonly occur, which unfortunately leaves only a minor fraction of funds for treatment, prevention, and research on addiction (Califano, 2009). Furthermore, a large fraction of individuals seeking treatment are being diagnosed by medical professionals who lack the necessary credentials or expertise to provide adequate evidence-based treatment (Lane, 2012).

Addiction can be defined as a "chronically relapsing disorder characterized by loss of control" over drug use, which affects drug intake and deregulates brain systems (Zorrilla, 2014). It can occur from the use of substances such as amphetamines and other stimulants, opioid narcotics, alcohol, and nicotine from tobacco products, among others. Drug addiction research has suggested acute rewarding effects are important to the initiation of drug use (Paulus, 2009). Acute drug dependence can occur after just a single episode of drug use, as measured by withdrawal symptoms as the drug is cleared from the system (Schulteis, 2010; Schulteis, 2008). As dependence progresses with repeated use, motivation to

continue taking drug may progressively shift towards self-medication to treat the withdrawal symptoms such as anxiety, or depression (Koob and Le Moal, 2006).

Amphetamines are one of the most common and well-studied substances linked to recreational abuse and addiction. Illicit use has always been prevalent, but since the 1990's amphetamine abuse has been on the rise particularly in young adults abusing prescription amphetamines (Berman et al, 2009). Common amphetamine medications are prescribed for attention deficit hyperactivity disorder (ADHD), narcolepsy, and obesity. Desired effects of amphetamines include resistance to fatigue, alertness, euphoria, elevation of mood, and suppression of appetite (Berman et al, 2009). Amphetamines are psychostimulants that mediate their behavioral effects by targeting neurotransmitters such as norepinephrine, dopamine, and serotonin, which are critical transmitters in the brain's arousal and reward circuitry (Advokat, 2007). It has been hypothesized that addiction to amphetamines and similar drugs involves a hijacking of the reward process within neural circuitry that includes critical parts of the limbic system that mediate reward and emotion, including the amygdala and nucleus accumbens (Hyman, 2005). This system contains neural networks that are involved in many complex functions, including memory and feelings of reward/pleasure/euphoria. Research has shown acute drug intake to decrease reward neurotransmission in these areas, and activate brain stress systems, both of which drive recurring drug use and lead to addiction via negative reinforcement, as the user attempts to overcome the negative emotional state during periods between drug intake (Koob and Le Moal, 2008).

Recently increasing evidence has pointed to a role for brain systems that mediate interoception as a critical component to further understanding drug addiction. The condition of "interoception" can be understood as the neuronal integration of an individual's entire physiological condition (Craig 2002). This is comprised of sensing the body's physiological condition, consciously representing the internal state of the individual, and producing the motivational action applicable to the situation (Craig, 2007). In this manner, the internal representation understood via interoception can function as an index of an individual's homeostatic levels (Craig, 2003).

Due to the ability of addictive drugs to alter the body's internal state and natural equilibrium, it is plausible to hypothesize that interoception can play a critical role in neuronal signaling that contributes to expression of drug reward and withdrawal. For example, it has been hypothesized that the extent to which a user will approach or avoid a drug is in response to how likely the external stimuli (i.e. the drug) will bring the individual back to its internal homeostasis (Paulus and Stein, 2010). Once addicted, an individual continues to abuse substances not only to experience the euphoria, but also to avoid any negative feelings. Additionally, it has been argued that interoception is a central aspect of many addiction relevant constructs such as arousal, stress, and reward (Paulus and Stein, 2013). Thus a deeper understanding of how information is integrated within the interoceptive network may provide further insight on drug addiction.

The anatomy of the interoceptive system is comprised of a complex network consisting of widespread afferent and efferent connections through

cortical, subcortical and limbic systems to coordinate multiple responses (Paulus, 2006). The information travels from the body's peripheral receptors through internal organs and ultimately converges on the caudal insular cortex. The caudal insula in turn has primary output to the rostral insular cortex, where interoceptive information is additionally integrated with input from supplementary system circuits such as cortical and limbic system structures that mediate reward/stress/emotion (Craig, 2002). These afferent/efferent pathways to and from the rostral insula connect it to: "(a) anterior cingulate cortex, which is important for cognitive control processes; (b) the amygdala, which is critical for processing stimulus salience; (c) the central striatum, central for the incentive motivational aspects of rewarding stimuli; and (d) the orbitofrontal cortex, which has been implicated in state-related valuation of external stimuli" (Paulus, 2009).

As a core integrative system for processing interoception, it has been suggested the insular cortex plays an important role in the development of drug craving, addiction, and the negative withdrawal state (Forget *et al.,* 2013). Neuroimaging studies have established that during sensory and emotional processes connected to interoception, the insula is activated (Craig 2003; Craig 2009). For example, a PET scan was able to detect and identify the insula as a very specific brain region for control and suppression of natural urges (Lerner *et. al*, 2009). Similarly, Contreras and colleagues demonstrated increased neuronal activity within the insular cortex when addicts are experiencing a drug craving in response to cues associated with drug use (Contreras *et al*., 2008). As reviewed in Paulus and Stewart 2013, additional fMRI imaging studies were able to

demonstrate drug-addicted users have a hyperactive insula cortex particularly during drug-specific conditions and reward-related mechanisms. For example, nicotine dependent users have enhanced insula activity during reward anticipation (Addicott et al., 2012). Similarly, users addicted to cocaine have a hyperactive insula cortex during reward anticipation, imagery related to stress, and also when cocaine-related cues are exhibited to the subject (Jia et al, 2011; Li et al., 2005; Bonson et al., 2002).

Direct causal evidence for a role of the insula in nicotine addiction was provided by Navqi and colleagues, who studied patients with damage to the insular cortex. It was found that smoking patients with damage that included critical portions of rostral insula were able to quit smoking more easily, and in general had less urge or craving to smoke, than those with no damage to the insula (Naqvi et al. 2007). This has been interpreted by some to suggest that when the insula is not functioning properly or has experienced damage, individuals are unable to identify the related emotional states and thus do not react in a typical manner to cues that might otherwise trigger the strong urge or craving to relapse (Gray and Critchley 2007). In support of this notion, a study conducted by Contreras and colleagues in rats demonstrated that a temporary "lesion" of the insula via local anesthetic inactivation could block the expression of amphetamine-conditioned place preference. Rats experiencing inactivation of the insula did not show a preference for a distinct environment previously paired with amphetamine, suggesting a direct role for caudal insula in conditioned associations with drug reward (Contreras et al, 2007).

However, it has been untested whether the insula plays a direct role in the *unconditioned*, direct rewarding effects of the drugs, as hypothesized by Paulus (Paulus et al., 2009). Thus further research is needed to more fully characterize its role of the insula in mediating distinct processes related to addiction, such as the direct rewarding effects of drugs that are important in the initiation of use, and the transition to dependence and loss of control over use that characterizes addiction. The aim of the present study was to determine whether reducing the function of the insular cortex regulates the direct reward-enhancing effects of drugs such as amphetamines, as measured by the ability of d-amphetamine to lower thresholds for electrical stimulation of the brain's reward pathways. The inhibition of the insular cortex in both the rostral insula (RI) and caudal insula (CI) via direct infusion of the local anesthetic bupivacaine was utilized to understand the role these regions play in a) the rewarding effects of brain stimulation through use of intracranial self-stimulation (ICSS) paradigm, and b) the effects of d-amphetamine on ICSS reward thresholds (Harrison et al, 2001; Kokkinidis et al, 1980; Paterson et. al, 2000).

2   Material and Methods

2.1 Animal Subjects

Male Wistar rats weighing 200-225 grams were purchased from Harlan Sprague Dawley (Livermore, CA). The rats were pair-housed and acclimated to the housing environment with a 12-hour light/dark cycle, lights on at 6:00am, prior to surgery and experimental testing.   Training and testing took place between 8:00am and 5:00pm Monday through Friday. The rats had *ad libitum* access to food and water. The Institutional Animal Care and Use Committee (IACUC) of the VA San Diego Healthcare System approved all experimental procedures.

2.2 Drugs

A 2% bupivacaine solution was prepared with artificial cerebral spinal fluid (aCSF) as the diluent; aCSF alone served as the vehicle control for intracranial infusions. D-amphetamine sulfate was dissolved in 0.9% physiological saline at a concentration of 0.5 mg/ml, and administered subcutaneously (SC) as 0.1 ml/100 g body weight for a dose of 0.5 mg/kg. Subcutaneous vehicle control injections consisted 0.9% physiological saline at a volume of 0.1ml/100g body weight.

2.3 Surgical Implantation of ICSS Electrode and Guide Cannulas

Rats were anesthetized using isoflurane (5% for induction, 1.5-2% for maintenance). The surgery site was shaved and then sterilized with alcohol and

betadine. The rat was then securely placed in a Kopf stereotaxic apparatus for implantation of the guide cannulas and stimulating electrode. The incisor bar of the instrument was placed at -3.3mm. Stainless steel cannulas (26 gauge, Plastics One, VA) measuring at 9mm and 12mm (RI and CI respectively) were implanted according to the coordinates described below, and then secured to the rat skull with dental acrylic. Stainless steel screws (Plastics one, VA) were also inserted in the skull to help secure the dental acrylic mount to the skull. Finally, a unilateral, bipolar electrode (Plastics One) targeted for the medial forebrain bundle was inserted, alternating left and right side of the rat brain, using bregma as a starting point at the coordinates: AP -2.8mm, ML +1.7/-1.7 (depending on left or right), and DV -7.9mm from the dura layer. After electrode insertion, additional dental cement was applied to anchor all parts of the guide cannula/electrode assembly to the skull surface and the anchor screws. After the surgery, local analgesic (2ml of 1% bupivacaine) and antibiotic ointments were applied to the surgery site. Furthermore, stylet occluders (SmallParts, 30 gauge) were inserted into each guide cannula after surgery, throughout recovery, and between infusions for maintenance of cannula patency. Typically, rats recovered from surgery over 5-7 days and then began training. Two distinct regions of the Insula Cortex were targeted via guide cannulas:

2.3.1. *Rostral Insular Cortex (RI)*

Using Paxinos and Watson Rat Brain Atlas (6th Edition), guide cannulas were aimed to enter at the following coordinates relative to bregma: +2.5mm anteroposterior, +/- 4mm medical lateral, -3.6mm

dorsoventral from the cranial surface. To perform an infusion an injector is inserted that extends 3 mm beyond the tip of each guide cannula, thus penetrating -6.6mm deep and ending at the targeted RI in the center of the agranular layer as shown in Figure 2.1.

Figure 2-1. RI Cannula and Injector Target

Schematic displaying location of cannula and injectors targeting the RI in the center of the agranular later (AP +2.5mm, ML +/-4mm, DV -3.6mm). GI, granular insula; DI, dysgranular insula; AID, agranular insula dorsal; AIV, agranular insula ventral; SIJ, second somatosensory cortex; LO, lateral orbital; rf, rhinal fissure. Image adapted to Paxinos and Watson (2007).

2.3.2. Caudal Insular Cortex (CI)

Using Paxinos and Watson Rat Brain Atlas (6th Edition), guide cannulas were aimed to enter at the following coordinates relative to bregma: -1.0mm anteroposterior, +/- 5mm medical lateral, -4mm dorsoventral from the cranial surface. To perform an infusion an injector is inserted that extends 3mm beyond the top of each guide cannula, thus penetrating -7mm deep and and ending at the targeted CI at the junction of the granular/dysgranular layer.

2.4 Inactivation of Insular Cortex

Prior to intracranial infusion and testing, rats were habituated to the infusion process through a series of so-called "mock" infusions. Inactivation of the insular cortex was performed with intracranial infusion of 2% bupivacaine. For vehicle groups, artificial CSF was utilized. After removing the stylets, injectors attached via tubing to a 10 ml Hamilton syringe were inserted into each guide cannula. Infusions of 0.3- 0.5 ml of 2% bupivacaine (or artificial CSF) were performed via the Harvard micro-infusion pump bi-laterally into the targeted regions. Rats receiving bupivacaine at 0.5ml were infused for 60 seconds and those receiving bupivacaine at 0.3ml were infused for 36 seconds. After the infusion, the injectors were left in place for an equivalent amount of time as the infusion process in order to allow for drug diffusion into the target tissue. After removing the injectors, the stylets were put back into place and the rat began testing approximately 5 minutes post-infusion.

2.5 Discrete-Trial Brain stimulation reward paradigm (ICSS)

Kornetsky and Esposito (1979) established the discrete-trial current intensity threshold technique of intracranial self-stimulation (ICSS), a psychophysical technique for determining brain stimulation reward thresholds. The surgically implanted electrode targets the medical forebrain bundle (MFB), which carries bundles of axons central to the reward circuit, thereby providing self-administered electrical brain stimulation to the animal. In the discrete-trial current-intensity ICSS paradigm rats must be trained through a series of levels referred to as CT1, CT2, and CT3. Throughout the training process, no drugs are being administered. The ICSS paradigm trains the rats to respond to a standard stimulation pulse of 250ms train duration and 100Hz sinusoidal wave current, with current intensity varied as described below. In the first level of ICSS training the rats must complete a quarter wheel turn response to receive a rewarding electrical stimulus. Reward is continuously available with no delay between receipt of one reward stimulus and availability of the next. Successful completion of the CT1 level entails 100 responses.

In CT2, there are sublevels with increasing time delays of 1, 3, 5, 10, and 15 seconds between reward opportunities. This level attempts to reinforce for only correctly timed responses. The rats receive a free non-contingent electrical pulse and have 7.5 seconds to respond. If the rat responds within 7.5 seconds of the non-contingent stimulus, this is considered a "positive response" and the rat receives a reward. If the rat does not respond within 7.5 seconds this is considered a "negative response", the intertrial interval (ITI) timeout begins after

a positive or negative response, and upon completion of the ITI another non-contingent pulse is delivered to the rat, beginning the cycle again. CT2 level completion entails at lease 40% positive response rate at each ITI (e.g. 40 or more positive responses within 7.5 sec, out of 100 trials).

The final stage of training allows determination of each rat's individual current intensity reward threshold with a series of ascending and descending current columns. To begin a session, the rat receives a non-contingent pulse of a given current intensity (established as one that reliably maintains responding at the CT1 and CT2 levels for a given rat). As in the CT2 level, the rat has 7.5 seconds to respond, followed by an ITI averaging 10 sec. Responses during the ITI reset the ITI, thus further delaying the next reward opportunity and encouraging low response rates, and ensuring that responding is not random, but rather is contingent upon perception of the "free" stimulation at a given current intensity. After 3 trials at the starting current intensity, the current is dropped successively in 5 mA steps as shown in Figure 2.5 below. The rats must respond within 7.5 sec to two out of three non-contingent stimuli to each current intensity to be considered a positive response at that given current intensity. Once a rat has failed to respond 2/3 times for two consecutive intensities, current intensity begins ascending again until the rat has responded at least 2/3 times at two consecutive intensities. This descending/ascending pattern is then repeated one additional time, for a total of 4 ascending/descending passes. The average current intensity above which a rat responded at least 2/3 times, and below which it did not, is calculated for each series, and the reward threshold is calculated as

the average of the 4 series values. Response latency is also measured as the latency between the non-contingent stimulus and the rat's response on all trials where a response occurred within the required 7.5 sec.
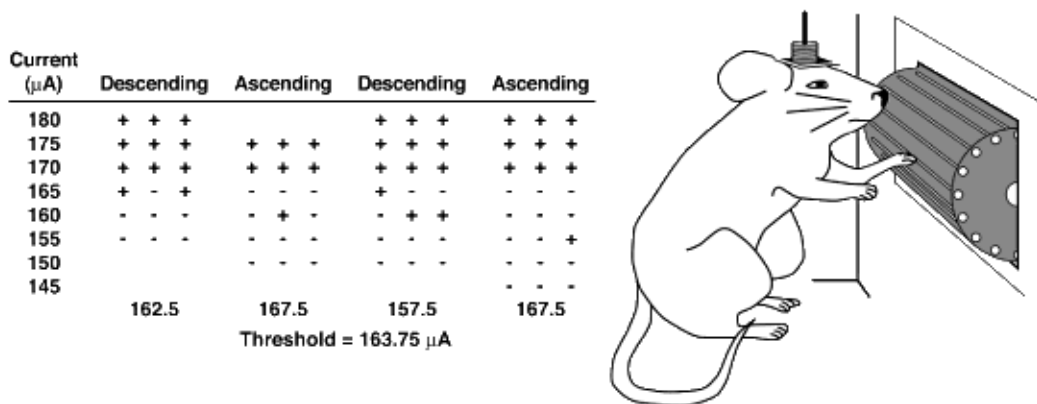

Figure 2-2. Discrete-Trial Brain Stimulation Reward Paradigm (ICSS)

This figure is a model of a hypothetical ICSS session. The image has been adapted from Neurobiology of Addiction (Koob and Le Moal, 2006)

2.6 Experimental Design

Wistar rats with ICSS electrodes and either RI or CI bilateral cannula implants were utilized in the experiments (n=98). After surgical implantation of the electrode and guide cannulas the rats were given adequate recovery time of at least five days before beginning the ICSS training process. After reaching the final level of ICSS training as described in Section 2.5 above, the rats received twice daily sessions until achieving a stabilized baseline stimulation threshold current in both a morning and afternoon session separated by 4 hours; stability was defined as less than 15% variation around mean threshold over at least 3 days. After achieving a stable baseline ICSS testing consists of a baseline phase and a test phase. The baseline phase consisted of three mock infusions as

described in Section 2.4 prior to each afternoon session, one per day, to habituate the rats to the handling and injection procedure of the test phase. A mock infusion was conducted by removing stylets from the guide cannula and gently holding the rat as one would during infusion, near the infusion pump while it is running for approximately one minute. The rat is then given a subcutaneous (SC) vehicle injection of saline and placed in the ICSS chamber to complete its afternoon session.

The testing phase was conducted once a stable threshold had been established under mock infusion conditions (again less than 15% variation from the mean of 3 days). On test day, the protocol involved the rat first receiving the intracranial infusion of either 2% bupivacaine or vehicle (aCSF). The rat is then place into its cage again for five minutes. Thereafter rats were given a subcutaneous vehicle or d-amphetamine (0.5 mg/kg) injection, placed into its cage for another five minutes, and then finally placed in the ICSS testing box chamber to complete a session. Immediately upon finishing the testing session, stylets are dropped.

2.7 Histological Verification of Surgical Cannula Placements

Directly following the completion of infusion and behavioral testing, stylets measuring +3mm longer than the guide cannula were inserted into the guide cannula (i.e. these stylets extend to the same depth as the injectors used to infuse bupivacaine). The stylets were left in position for at least five days to ensure the formation of a tract the same length as the injector that would be

visible upon histological examination. After 5 days the animals were given a 2ml intraperitoneal (IP) injection of Euthasol and perfused transcardially by a 10% formalin solution. Animal brains were removed and fixed in a 10% formalin solution for a 24 hour time period. The brains were then switched into a 30% sucrose phosphate buffered saline (PBS) solution for approximately three days or until the brain had fully sunk within the vial. Thereafter, the brains were removed from solution, frozen in optimum cutting temperature (OCT) gel and then coronally sliced at a width of 50mm via a cryostat. The brain slices were preserved on slides and stained using cresyl violet for verification of proper surgical cannula placement.

The Rat Brain in Stereotaxic Coordinates by Paxinos and Watson (2007) brain atlas was utilized as a guide for analyzing the injector and cannula placements. Animals with injectors clearly terminating within the region of the insular cortex were included in the study. For CI surgical cannula placement, the injectors must terminate bilaterally within the caudal granular or dysgranular insular cortex. This region includes anteroposterior -0.8 to -1.2mm behind bregma (Figure 2-3). The region is bordered dorsally by the somatosensory cortex and ventrally by the agranula insular cortex as well as the rhinal fissure. For the RI surgical cannula placement, the injectors must terminate bilaterally within the rostral agranular insular cortex. This region includes anteroposterior +2.28 to +2.8 in front of bregma (Figure 2-4). The region is bordered dorsally by the dysgranular insular cortex as well as ventrally by the rhinal fissure.
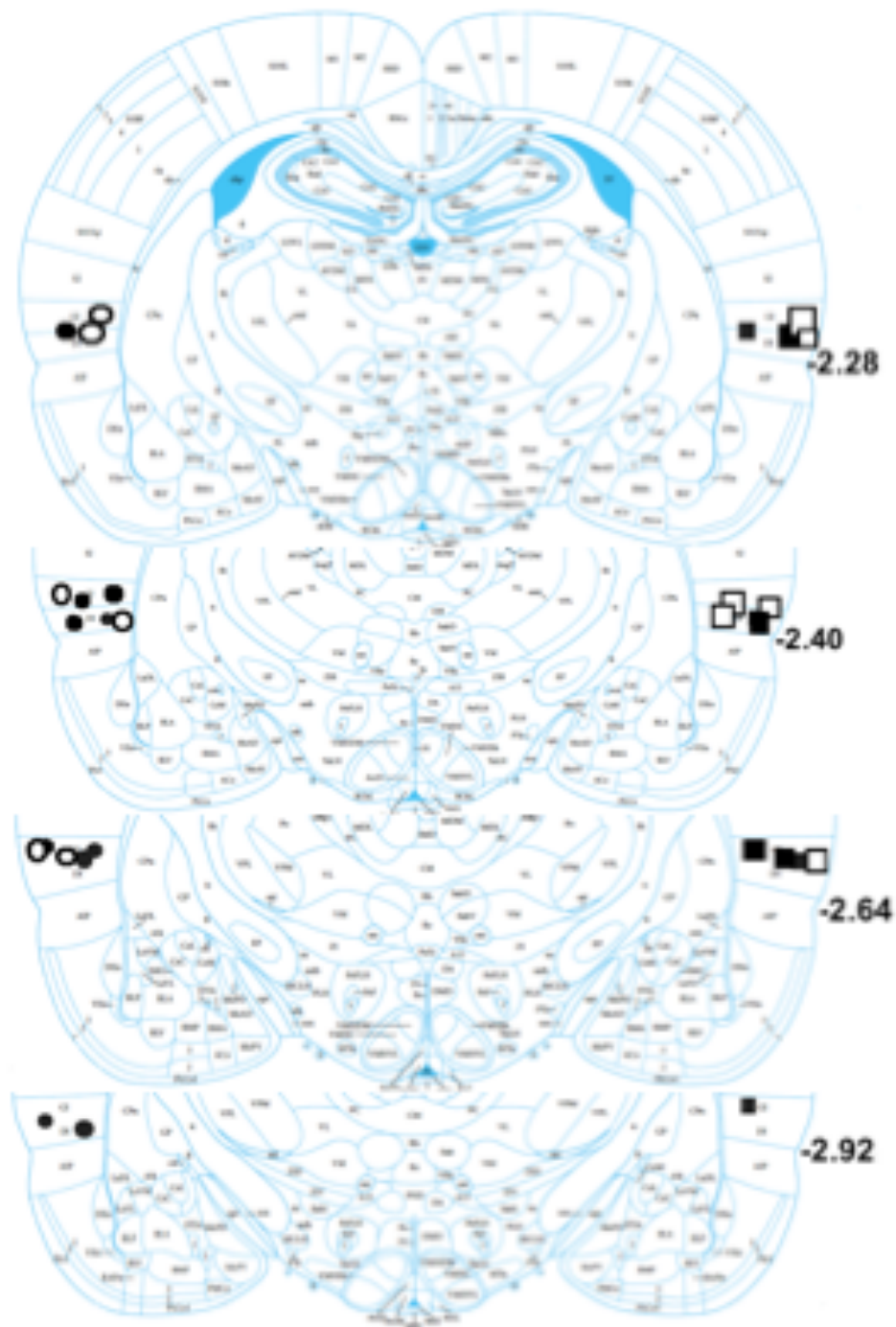
Figure 2-3. CI Histology Verification for Cannula Placement and Drug Infusion

Injector Placement for CGIC infusions. Numbers represent distance from bregma, anteroposterior. Symbols represent location of injector termination. Closed circles represent Veh-Veh. Open circles represent Veh-Amph. Closed squares represent Bup-Veh. Open squares represent Bup-Amph. Images adapted from the atlas of Paxinos and Watson (6th edition).
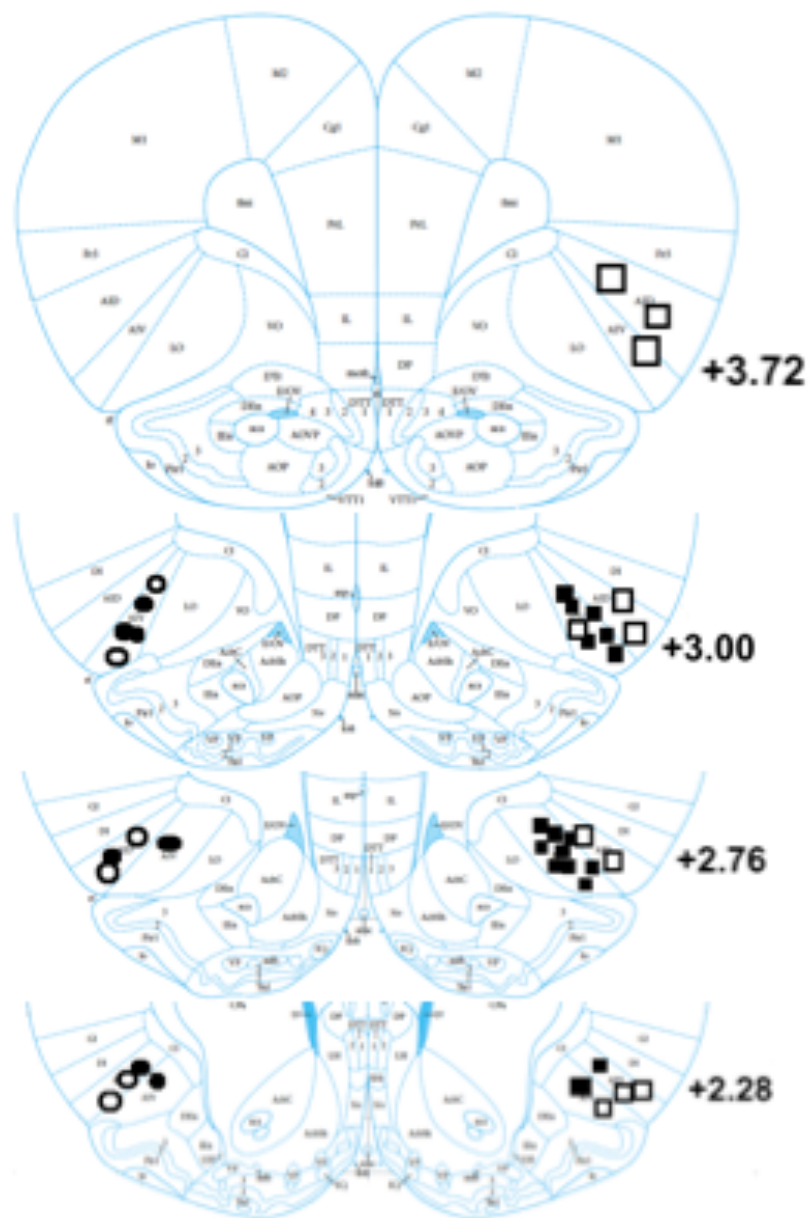
Figure 2-4. RI Histology Verification for Cannula Placement and Drug Infusion

Injector Placement for RAIC infusions. Numbers represent distance from bregma, anteroposterior. Symbols represent location of injector termination. Closed circles represent Veh-Veh. Open circles represent Veh-Amph. Closed squares represent Bup-Veh. Open squares represent Bup-Amph. Images adapted from the atlas of Paxinos and Watson (6th edition).

2.8 Data Analysis

The data on test day (threshold, response latency) were expressed as a percentage of the average baseline values from mock infusion sessions (see Table 2-1 for the raw baseline threshold and latency means for each experimental group). Statistical analysis was performed using ANOVAs through the JMP Software program, followed by individual means comparisons as appropriate using the Bonferroni correction to hold experiment-wise error to a constant level of $p < 0.05$.

Table 2-1. Bupivacaine and Amphetamine Drug Summary

Summary of drug combinations, baseline thresholds, and baseline latency for all RI and CI groups in research study. Abbreviations are as follows: Veh= Vehicle, Bup 0.3= Bupivacaine 0.3ml, Bup0.5= Bupivacaine 0.5ml, and Amph0.5= Amphetamine 0.5ml.

Surgery Type	Drug Combination (IC-SC)	Sample Size	Baseline Threshold (mA)	Baseline Latency (sec)
RI	Veh-Veh	n=10	86.05+/- 13.59	3.07 +/- 0.12
	Bup0.3-Veh	n=9	92.61 +/- 9.47	2.62 +/- 0.15
	Bup0.5-Veh	n=10	66.09 +/- 9.72	2.50 +/- 0.10
	Veh-Amph0.5	n=8	109.38 +/- 24.53	2.67 +/- 0.19
	Bup0.3-Amph0.5	n=8	103.32 +/- 15.91	2.82 +/- 0.15
	Bup0.5-Amph0.5	n=6	92.71 +/- 9.59	2.87 +/- 0.16
	Diffusion Control (Bup0.5-Veh)	n=7	91.65 +/- 12.61	2.82 +/- 0.20
CI	Veh-Veh	n=10	89.73 +/- 6.89	2.75 +/- 0.18
	Bup0.5-Veh	n=10	90.01 +/- 9.17	2.79 +/- 0.11
	Veh-Amph0.5	n=10	90.42 +/- 9.28	2.97 +/- 0.21
	Bup0.5-Amph0.5	n=10	94.00 +/- 13.00	2.72 +/- 0.16

3 Results

3.1 Experiment 1: Effects of Bupivacaine Alone on Brain Reward Thresholds.

As shown in Figure 3-1, rats infused with bupivacaine in the RI but not CI appeared to have elevated thresholds relative to aCSF-infused vehicle controls. This was confirmed in a 2x2 ANOVA with brain region (RI, CI) and bupivacaine condition (Bup0.5, Veh), with a significant main effect of brain region ($[F(1,36)=14.30$, $P<0.0006]$); the region x condition interaction approached but did not quite achieve statistical significance ($[F(1,36)=3.76$, $P=0.0605]$). As displayed in Figure 3-2 there was no effect of bupivacaine on response latency, regardless of injection site; neither main effect (region, drug condition) nor the interaction reached significance (all F's < 0.92, p's > 0.30).

Given the significant main effect of region, a lower volume of bupivacaine was infused into the RI to determine an ineffective volume of infusion for use in the amphetamine study. As shown in Figure 3-1, bupivacaine volume-dependently elevated thresholds of rats infused in the RI. This was confirmed by a 1-factor ANOVA ($[F(2,27)=3.65$ $P<0.04]$. Follow-up analysis with a diffusion control group revealed that while the group infused with Bup0.5 into the RI was significantly different from the group infused into the RI with Bup0.3 ul ($[F(1,27)=7.03$ $P<0.02]$); the diffusion control group was not statistically different from Bup0.5 RI groups ($[F(1,27)=3.11$, $P=0.09$), nor Bup0.3 RI groups $[F(1,27)=0.99$, $P=0.33]$, suggesting regional specificity of effect at the Bup0.5 volume infused into the RI but not dorsal to it.
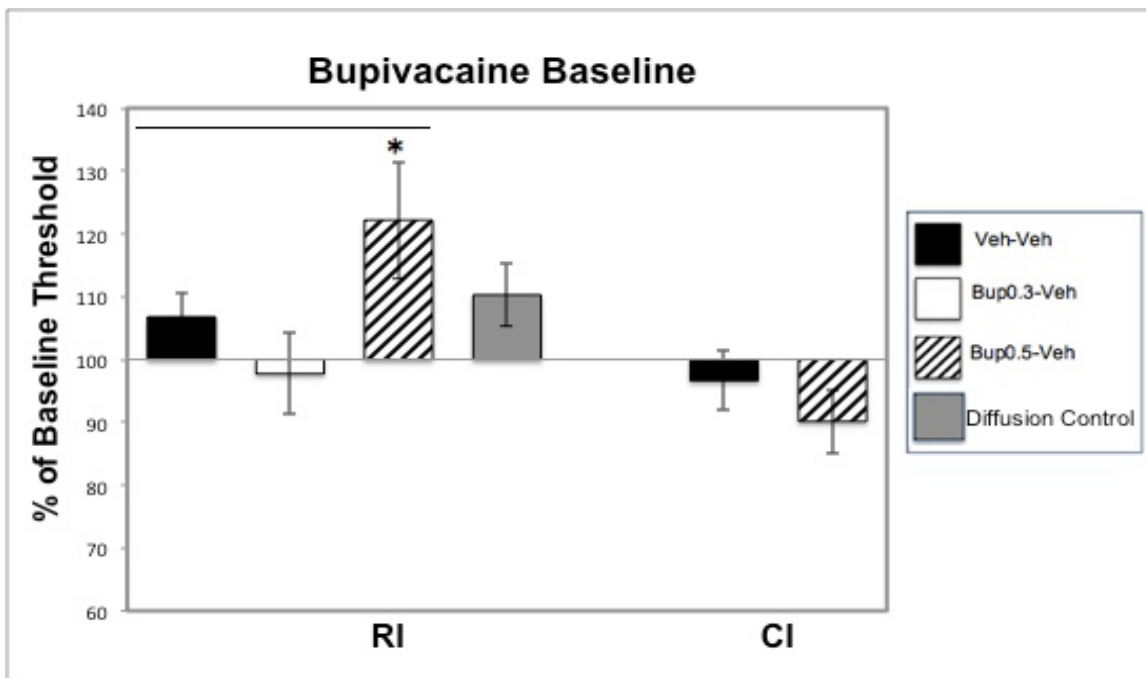
Figure 3-1. Bupivacaine Baseline

This graph displays the % baseline threshold of RI and CI bupivacaine dose response. Baseline thresholds were presented as mean (+/-SEM). The main effects of bupivacaine dose response were found in the RI groups: Bup0.5 v Bup0.3 was significant, Bup0.3 v DC not significant, and Bup0.5 v DC not significant.
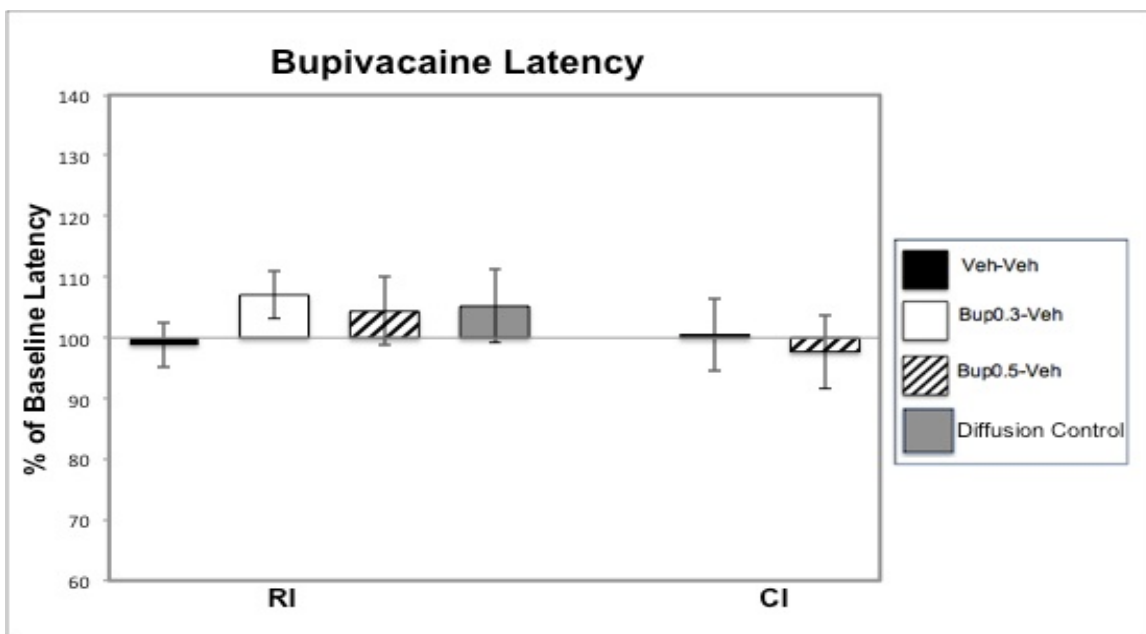

Figure 3-2. Bupivacaine Latency

This graph displays the bupivacaine latency for all the RI and CI groups and results were measured as a % of baseline threshold. Baseline thresholds were presented as mean (+/-SEM). The effect of bupivacaine on latency did not reach significance in either brain region.

3.2 Experiment 2: Effects of Insular Inactivation on Amphetamine-Enhanced Reward Thresholds

In CI, the effect of amphetamine was the same regardless of whether bupivacaine or vehicle was infused IC, (Figure 3-3), as revealed in a 2x2 ANOVA by a significant main effect of amphetamine condition [$F_{(1,35)}=70.34$, $P<0.0001$]) but no main effect of IC infusion condition (Veh vs Bup0.5, [$F_{(1,35)}=3.43$, $P=0.073$]) or IC x amphetamine interaction ([$F_{(1,35)}=0.07$, $P=0.79$]). The effect on latency approached but did not reach significance ([$F_{(1,35)}=4.05$, $P=0.0519$]), see Figure 3-4.
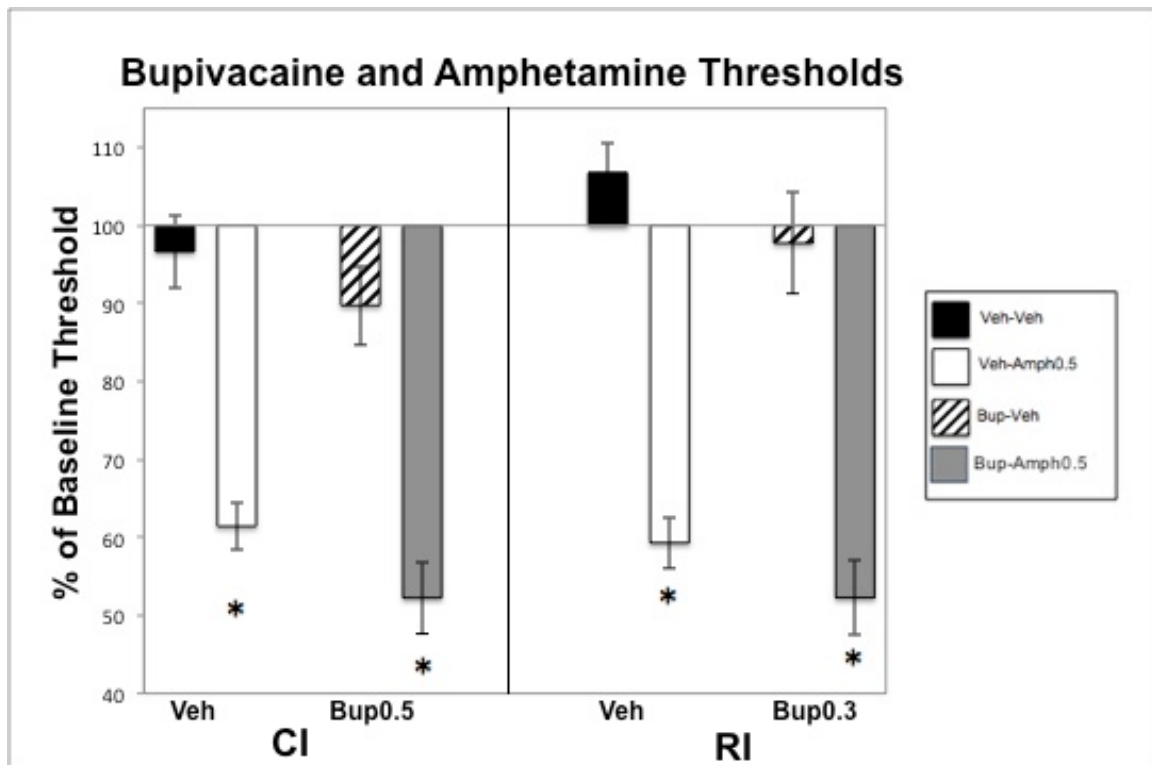

Figure 3-3. Bupivacaine and Amphetamine Thresholds

CI groups received Veh-Veh, Veh-Amph0.5, Bup0.5-Veh, and Bup0.5-Amph0.5 drug combinations and results were measured as a % of baseline threshold. Baseline thresholds were presented as mean (+/-SEM). RI groups received Veh-Veh, Veh-Amph0.5, Bup0.3-Veh, and Bup0.3-Amph0.5 drug combination and results were similarly measured as a % of baseline threshold. Amphetamine produced a significant main effect; the decrease in threshold was the same in both CI and RI whether bupivacaine or vehicle was infused.

A similar pattern was seen with Bup0.3 vs Vehicle in RI (Figure 3-3), with a significant main effect of amphetamine ([$F(1,31)=106.08$, $P<0.0001$]) but no main effect of IC condition (Veh, Bup0.3, [$F(1,31)=3.18$, $P=0.084$]) or interaction ([$F(1,31)=0.05$, $P=0.83$]). The main effect of amphetamine on latency, shown in Figure 3-4, was significant [$F(1,31)=5.94$, $P<0.0208$]), with both vehicle and Bup0.3 infused groups treated with amphetamine showing reduced latencies relative to SC vehicle groups.
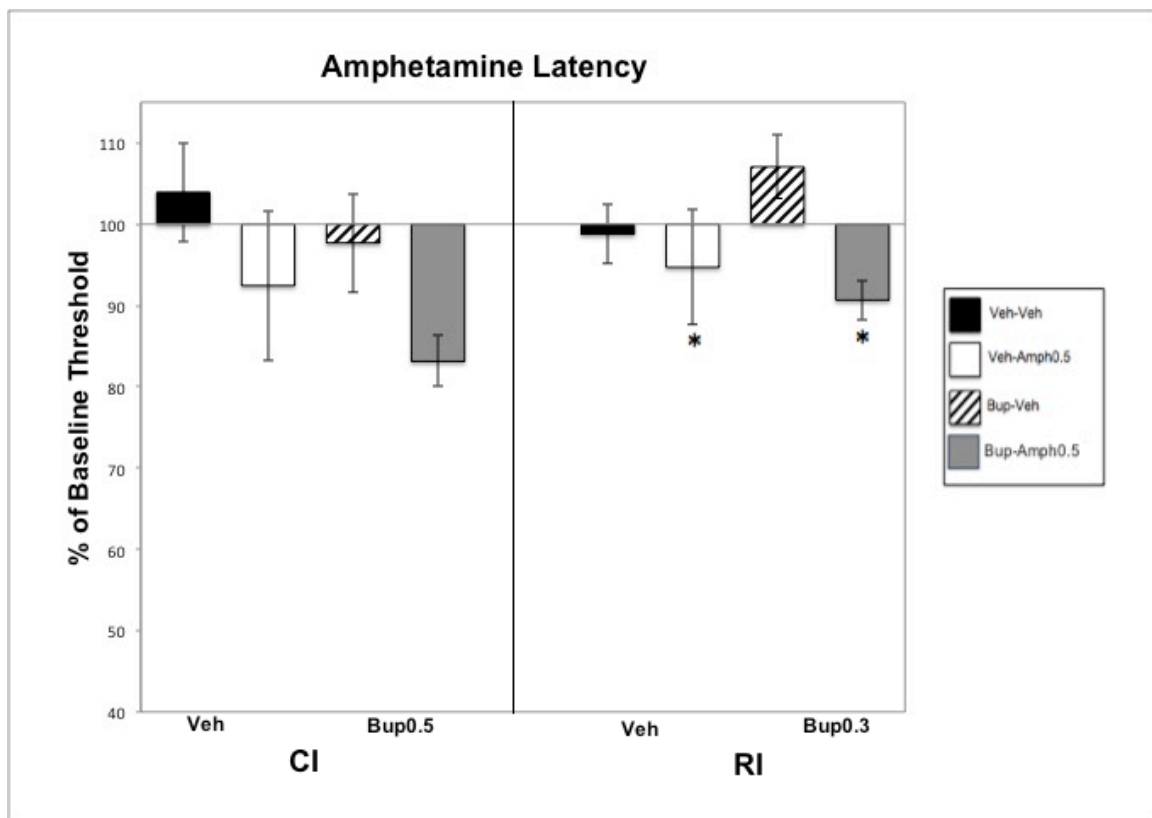

Figure 3-4. Amphetamine Latency

CI groups received Veh-Veh, Veh-Amph0.5, Bup0.5-Veh, and Bup0.5-Amph0.5 drug combinations and results were measured as a % of baseline threshold. Baseline thresholds were presented as mean (+/-SEM). The effect on latency approached but did not quite reach statistical significance. RI groups received Veh-Veh, Veh-Amph0.5, Bup0.3-Veh, and Bup0.3-Amph0.5 drug combinations and results were measured as a % of baseline threshold. In this graph, the RI amphetamine latency in groups infused with amphetamine for both vehicle and Bup0.3 displayed significant differences from the SC vehicle groups.

4   Discussion

The intracranial self-stimulation (ICSS) paradigm was utilized to measure brain reward current intensity thresholds to assess the role of the insular cortex in the rewarding effects of brain stimulation, and in the reward-enhancing effects of an abused drug such as d-amphetamine. The role of RI and CI in these reward-related processes was examined with intracranial infusion of the local anesthetic bupivacaine, which inhibits axonal transmission via binding to and blocking intracellular sodium channels, thus preventing depolarization (Wagner, 2014). Inactivation of the RI, but not the CI, in the absence of amphetamine volume-dependently elevated reward thresholds, as shown in Figure 3-1.

In order to verify the effects found were attributable specifically to inactivation of the RI and not due to diffusion of bupivacaine to nearby brains regions, a group of RI diffusion control rats were tested with cannula that terminated 1mm above the normal site. Sites dorsal to the injection location are typically chosen as diffusion control sites because diffusion up the injector tract represents the path of least resistance for diffusion away from the target region. The threshold-elevating effects of the 0.5 ml volume of bupivacaine were found to be region-specific, with infusions of bupivacaine dorsal to the rostral agranular region not replicating effect of infusions directly into rostral agranular regions. This regionally specific effect of inactivating the RI suggests that neuronal activity in the RI may modulate reinforcing effects of electrical stimulation of the medial forebrain bundle. The fact that this modulatory effect was not seen upon infusion of bupivacaine into the CI is perhaps not surprising, given the extensive

reciprocal connections of RI with circuitry critical to reward (i.e basolateral amygdala regulating attention, the nucleus accumbens controlling reward/motivation and the prefrontal cortex regulating cognitive behavior and decision making) (Craig 2002; Craig 2003).

Our results with brain stimulation reward can be contrasted with the results of Wolfe (2011), who demonstrated that inactivation of either the RI or CI produced increased anxiety-like effects in multiple behavioral paradigms (Wolfe, 2011). Wolfe's results were also region-specific, with infusions dorsal to RI or CI not producing any significant effects. The anxiety-like effects of inactivation of either CI or RI could be attributable to an inability of an animal to properly assess a novel fear-inducing situation like the elevated plus maze in the absence of interoceptive signals processed sequentially through CI and then RI. In contrast, interruption of the interoceptive signal at the caudal level of insular cortex did not alter brain stimulation reward in our study, suggesting that the interoceptive inputs entering the insula through CI may be less critical to the direct rewarding effects of brain stimulation than the afferent/efferent connections of RI to other reward-related regions. The exact connections mediating this modulatory effect of RI activity on brain reward remain to be elucidated.

The second part of this thesis evaluated the effects of insular inactivation with bupivacaine on amphetamine enhancement of reward as measured by a lowering of current intensity thresholds in the same ICSS paradigm. As expected due to previous literature on the rewarding properties of acute amphetamine use, amphetamine significantly decreased reward thresholds and this effect was

similarly seen in vehicle control groups infused with aCSF into RI and CI, see Figure 3-3 (Schaefer and Michael, 1988). At low volumes of bupivacaine that do not produce significant changes in threshold on their own, the effect of amphetamine remained unchanged following bupivacaine inactivation of RI and CI. Consistent with its reward-enhancing and stimulant effects, amphetamine significantly decreased thresholds and shortened response latency, although the latter effect did not quite reach significance in rats in the CI infusion experiment. These results suggest the stimulant and rewarding effects of amphetamine at a 0.5 mg/kg dosage do not critically depend on neuronal activity in either the anterior nor the posterior region of the insula cortex. The findings with the stimulant (latency reducing) effects of amphetamine are not surprising, since motor-stimulant effects of amphetamines are well-characterized to depend upon the dorsal and ventrial striatum (Everitt, 2002), but Paulus and colleagues have postulated that the rewarding effects of abused drugs may rely upon interoceptive processing in the insula (Paulus, 2014), a hypothesis that is not supported by the present data. However, it must be noted that only one dosage for the amphetamine was tested, and that the effects at this dose are near-maximal as measured by reduction of current intensity thresholds in our ICSS paradigm (Schulteis, unpublished results), and more definitive support for this conclusion would be provided by further studies examining additional amphetamine doses to determine whether inactivation of RI or CI may shift the amphetamine dose-effect function for reward enhancement. Nonetheless, based on the current data, in combination with work of Contreras and colleagues (2007)

using the place preference conditioning paradigm, our initial conclusion is that the insular cortex may play a more critical role in the conditioned rather than the direct/unconditioned rewarding effects of amphetamine.

References

1. Addicott, M.A., Baranger, D.A., Koznik, M.J., Smoski, G.S., McClernon, F.J. (2012). Smoking withdrawal is associated with increases in brain activation during decision making and reward anticipation: a preliminary study. Psychopharmacology, 563-573.

2. Advokat, C. (2007). Update on amphetamine neurotoxicity and its relevance to the treatment of ADHD. J Atten Disord, 8-16.

3. Berman, Steven. Kuczenski, Ronald, London, Edythe. (2009). Potential Adverse Effects of Amphetamine Treatment on Brain and Behavior: A Review. Mol Psychiatry, 123-142.

4. Bonson, K.R., Grant, S.J., Contoreggi, C.S., Links, J.M., Metcalfe, J, Weyl, H.L., Kurian, V, Ernst, M, London, E.D. (2002). Neural systems and cue-induced cocaine craving. Neuropsychopharmacology, 376-386.

5. Califano, Joseph. (2009). Shoveling Up II: The Impact of Substance Abuse on Federal, State, and Local Budgets. The National Center on Addiction and Substance Abuse at Columbia University.

6. Contreras, M, Ceric, F, Torrealba, F. (2008). The negative side of emotions: addiction to drugs of abuse. Rev Neurol, 471-476.

7. Craig,AD. How do you feel? Interoception: the sense of the physiological condition of the body. Nat. Rev Neurosci. 2002;3: 655-666.

8. Craig,AD. Interoception: the sense of the physiological condition of the body. Curr. Opin. Neurobiol. 2003;13: 500-505.

9. Craig AD. Interoception and emotion: a neuroanatomical perspective. In: Lewis M, Haviland-Jones JM, Feldman Barrett L, editors. Handbook of Emotions. New York: Guilford Press; 2007. p. 272–90.

10. Everitt, B and Wolf, M. (2002). Psychomotor Stimulant Addiction: A Neural Systems Perspective. The Journal of Neuroscience, 3312-3320.

11. Forget, B, Pushparaj, A, Le Foll, B. (2010). Granular insular cortex inactivation as a novel therapeutic strategy for nicotine addiction. Biological Psychiatry, 26-271.

12. Gray,MA, Critchley,HD. Interoceptive basis to craving. Neuron 2007;54: 183-186.

13. Harrison AA, Liem YT, Markou A. (2001). Fluoxetine combined with a serotonin-1A receptor antagonist reversed reward deficits observed during nicotine and amphetamine withdrawal in rats. Neuropsychopharmacology, 55-71.

14. Hoyert, Donna and Xu, Jiaquan. (2012). Deaths: Preliminary Data for 2011. National Vital Statistics Reports.

15. Hyman,SE. Addiction: a disease of learning and memory. Am. J Psychiatry 2005;162: 1414-1422.

16. Jia, Z., Worhunsky, P.D., Carroll, K.M., Rounsaville, B.J., Stevens, M.C., Pearlson, G.D., Potenza, M.N. (2011). An initial study of neural responses to monetary incentives as related to treatment outcome in cocaine dependence. Biol. Psychiatry, 553-560.

17. Kokkinidis L, Zacharko RM, Predy PA. (1980). Post-amphetamine depression of self-stimulation responding from the substantia nigra: reversal by tricyclic antidepressants. Pharmacol Biochem Behav, 379-83.

18. Koob, G. F., and Le Moal, M. (2006). Neurobiology of addiction, (Amsterdam ; Boston: Elsevier/Academic Press).

19. Koob, G. F., and Le Moal, M. (2008). Neurobiological mechanisms for opponent motivational processes in addiction. Philosophical Transactions of The Royal Society.

20. Lane, Jeffrey (2012). Addiction Medicine: Closing the Gap between Science and Practice. The National Center on Addiction and Substance Abuse at Columbia University.

21. Lerner,A, Bagic,A, Hanakawa,T, Boudreau,EA, Pagan,F, Mari,Z, Bara-Jimenez,W, Aksu,M, Sato,S, Murphy,DL, Hallett,M. Involvement of insula and cingulate cortices in control and suppression of natural urges. Cereb. Cortex 2009;19: 218-223.

22. Li, C.S., Kosten, T.R., Sinha, R. (2005). Sex differences in brain activation during stress imagery in abstinent cocaine users: a functional magnetic resonance imaging study. Biol. Psychiatry 487-494.

23. Naqvi,NH, Rudrauf,D, Damasio,H, Bechara,A. Damage to the insula disrupts addiction to cigarette smoking. Science 2007;315: 531-534.

24. Paterson, NE, Myers, C, Markou, A. (2000). Effects of reoeated withdrawal from continuous amphetamine administration on brain reward function in rats. Psychopharmacology, 440.

25. Paulus,MP, Stein,MB. An insular view of anxiety. Biol. Psychiatry 2006;60: 383-387.

26. Paulus,MP, Stein,MB. Interoception in anxiety and depression. Brain Struct. Funct. 2010;214: 451-463.

27. Paulus, MP, Stewart, JL. (2014). Interoception and drug addiction. Neuropharmacology.

28. Paulus,MP, Tapert,SF, Schulteis,G. The role of interoception and alliesthesia in addiction. Pharmacol Biochem. Behav. 2009;94: 1-7.

29. Paxinos, G., and Watson, C. (2007). The Rat Brain in Stereotaxic Coordinates, 6 edn (San Diego: Academin Press).

30. Schaefer, G.J., Micahel, R.P. (1988). An analysis of the effects of amphetamine on brain self-stimulation behavior. Behav Brain Res., 93-101.

31. Schulteis, Gerhard. (2010). Brain Stimulation and Addiction. Encyclopedia of Behavioral Neuroscience 2010, 209-220.

32. Wagner, M, Zausig, YA, Ruf, S, Rudakova, E, Gruber, M, Graf, BM, Volk, T. (2014). Lipid rescue reverses the bupivacaine-induced block of the fast Na+ current (INa) in Cardiomyocytes of the rat left ventricle. Anesthesiology, 724-36.

33. Wolfe, Monica. (2011). Inactivation of the Insular Cortex Increases Anxiety-Like Behavior in Rats: Relevance to Drug Abuse and Interoception. UCSD.

34. Zorrilla, E., Logrip, M., Koob, G. (2014). Corticotrpin releasing factor: A key role in the neurobiology of addiction. Front Neuroendocrinology.