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Psychobiological Mechanisms of Maladaptive Cocaine Seeking

Dana Conlisk

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THÈSE PRÉSENTÉE

POUR OBTENIR LE GRADE DE

**DOCTEUR DE
L'UNIVERSITÉ DE BORDEAUX**

ÉCOLE DOCTORALE « SCIENCES DE LA VIE ET LA SANTÉ »

SPÉCIALITÉ NEUROSCIENCES

Par Dana Elizabeth CONLISK

**PSYCHOBIOLOGICAL MECHANISMS OF
MALADAPTIVE COCAINE SEEKING**

Sous la direction de : Véronique DEROCHE-GAMONET

Soutenue le 14 Septembre 2022

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Bisous,



ABSTRACT

About 20% of cocaine users suffer with severe cocaine use disorder (CUD) or addiction, and to date, there are limited therapeutic options available. Due to this, there is an increased need to focus on the neurobiology surrounding key behavioral features of cocaine use disorder.

My thesis focuses on understanding the psychobiological mechanisms of the emergence of maladaptive seeking- a key behavioral feature in those who suffer from CUD. Within my research, I use a model of CUD in the rat previously pioneered by our lab and acknowledged as a breakthrough in the field. In this model, 15% of rats develop an addiction-like behavior after protracted intravenous self-administration (SA). Critically, after early cocaine SA, a maladaptive seeking behavior emerges that is reliably predictive of the transition to addiction.

The factors influencing the emergence of this maladaptive seeking behavior are explored in this manuscript.

Combining behavioral methods, analytical chemistry and calcium imaging, I study the psychopharmacological and neurobiological determinants of this early maladaptive seeking behavior. I hypothesized and demonstrated that early maladaptive seeking results from an increased control of behavior by incentive internal cues at the expense of control by adaptive inhibitory external cues, which is related to altered recruitment of the prelimbic cortex (PL). Addiction is a relapsing disorder with 80% of users failing at remaining abstinent, making relapse a major clinical challenge. Therefore, I focused on how the mechanisms identified in the first two objectives modulate relapse, modeled in rats using the reinstatement procedure.

This PhD work explores and identifies crucial psychopharmacological mechanisms that are involved in a key behavioral characteristic of the transition to addiction: maladaptive seeking. In addition, it detects neurobiological correlates of predictive markers of the vulnerability to addiction. Consequently, it sets an early foundation to explore potential treatment modalities that piggyback off of the psychopharmacological or neurobiological differences discovered.

Keywords: cocaine use disorder, self-administration, vulnerability, animal model, prelimbic cortex, fiber photometry, outbred rats

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TABLE OF CONTENTS

Index of figures.....	9
Index of tables.....	14
List of abbreviations.....	15
Preface.....	16
Introduction.....	18
Scientific context.....	20
Cocaine use disorders (CUD).....	20
Economic and Social Burden of Problematic Cocaine Use.....	20
Diagnostic Criteria of Problematic Cocaine Use.....	20
Maladaptive Drug Use, the Addiction Cycle, and Craving.....	22
Current Treatments for Problematic Cocaine Use.....	22
Animal models of CUD: Toward measuring maladaptive cocaine self-administration.....	24
Modelling maladaptive self-administration behavior.....	24
Psychobiological features of maladaptive cocaine self-administration.....	28
Maladaptive behavioral inhibition and discrimination.....	28
Maladaptive behavioral inhibition and cocaine PK/distribution.....	29
Neurobiology of maladaptive cocaine self-administration: focus on the Prelimbic cortex and the prefrontal to Nucleus Accumbens Core pathway.....	30
Prelimbic cortex.....	30
Prelimbic/nucleus accumbens core pathway and balance between response to incentive and adaptive inhibition.....	31
Susceptibility of the PL-NacC pathway to cocaine PK/distribution and relation to addiction vulnerability.....	32
Influence of sex- hormonal influences.....	33
Vulnerability to addiction.....	33
Cocaine pharmacokinetics and pharmacodynamics.....	34
Neurobiology.....	34
General hypothesis and objectives.....	35
Common materials and methods.....	38
Surgery.....	38
Drugs.....	38

Intravenous self-administration apparatus.....	38
Intravenous self-administration procedure	39
Data and statistical analysis	40
Chapter 1: Psychopharmacological contributions to maladaptive cocaine seeking.....	42
Materials and methods	43
Subjects	43
Specific intravenous self-administration procedure	43
Experimental timeline	46
Adaptive inhibition as a function of drug state	46
Adaptive inhibition over incented cocaine seeking	47
Specific data and statistical analysis.....	48
Results	50
Adaptive inhibition as a function of drug state.....	50
Manipulation 1: Effect of non-contingent cocaine injections on maladaptive seeking.....	50
Manipulation 2: Effect of extending the duration of the no drug period session on initiation of maladaptive seeking.....	60
Manipulation 3: Effect of a drug free state on maladaptive seeking expression	63
Adaptive inhibition over incented cocaine seeking	69
Manipulation 4: Effect of adaptive inhibitory discriminative stimulus (no drug DS) on cocaine-induced reinstatement.....	69
Discussion	78
Chapter 2: Pharmacokinetic influences on maladaptive cocaine seeking	82
Materials and methods	85
Subjects	85
Specific drugs.....	85
Specific surgery.....	85
Specific intravenous self-administration procedure	86
Brain collection procedure	86
Brain punch procedure.....	86
Cocaine analysis procedure.....	87
Blood collection procedure for plasma butyrylcholinesterase assay.....	87
Butyrylcholinesterase activity measurement.....	88

Experimental timeline	88
Specific data and statistical analysis.....	88
Results	90
Brain cocaine and metabolites: relationship with self-administration behavior.....	90
Plasma butyrylcholinesterase activity: relationship with self-administration behavior.....	105
Discussion.....	109
Chapter 3: Prelimbic correlates of maladaptive cocaine seeking	116
Introduction.....	116
Materials and methods	117
Subjects	117
Specific surgery.....	117
Fiber photometry system	118
Coupling of fiber photometry and self-administration systems	118
Specific intravenous self-administration procedure	119
Histology.....	122
Experimental timeline	122
Specific data and statistical analysis.....	123
Results	127
Pyfiber development	127
Principle.....	127
Example of application	130
Prelimbic activity and maladaptive seeking expression.....	133
Manipulation 1: PL recruitment by an adaptive inhibitory discriminative stimulus (no drug DS)- relationship with active no drug responding	133
Manipulation 2: PL recruitment by an adaptive inhibitory discriminative stimulus (no drug DS)- relationship with active no drug responding as a function of drug state	136
Manipulation 3: PL activity during no drug period as a function of active no drug responding... ..	142
Manipulation 4: PL activity during no drug period as a function of drug state- effect of non- contingent cocaine injections during no drug period	143
Discussion.....	145
Conclusion and perspectives	148
Conclusion	148

Perspectives..... 150
Bibliography..... 153

INDEX OF FIGURES

FIGURE 1: SELF-ADMINISTRATION APPARATUS- OPERANT CHAMBERS WERE EQUIPPED WITH TWO HOLES AT OPPOSING SIDES OF THE CHAMBER. THE CONDITIONED STIMULUS (COCAINE CS) WAS LOCATED ABOVE THE ACTIVE HOLE WHILE THE DRUG DISCRIMINATIVE STIMULUS (DRUG DS) WAS LOCATED ON THE WALL WHERE THE INACTIVE HOLE WAS LOCATED. THE NO DRUG DISCRIMINATIVE STIMULUS (NO DRUG DS) WAS LOCATED ON THE CEILING OF THE CHAMBER. COCAINE INJECTIONS WERE DONE BY ACTIVATION OF A PUMP LOADED WITH A SYRINGE CONTAINING A COCAINE SOLUTION SUITABLE FOR IV DELIVERY. THE PUMP WAS CONNECTED TO THE RAT BY SILASTIC TUBING (ENCASED IN METAL) RUNNING FROM THE SYRINGE TO THE CATHETER PORT ON THE SCAPULAR REGION OF THE RAT. CREATED WITH BIORENDER.COM. 39

FIGURE 2: SELF-ADMINISTRATION SESSIONS- A. BASELINE (OR BASAL) SELF-ADMINISTRATION SESSIONS CONSISTED OF THREE FORTY-MINUTE DRUG PERIODS SEPARATED BY TWO FIFTEEN MINUTE NO DRUG PERIODS. B. A NON-CONTINGENT INJECTION SESSION WAS A BASAL SELF-ADMINISTRATION SESSION APART FROM THE FIRST NO DRUG PERIOD WHERE TWO UNSIGNALLED NON-CONTINGENT INJECTIONS OCCURRED AT THE FIFTH AND TENTH MINUTE OF THE FIRST NO DRUG PERIOD. C. THE LONG NO DRUG SESSION CONSISTED OF A NORMAL 40 MIN DRUG PERIOD FOLLOWED BY A 90 MINUTE NO DRUG PERIOD RATHER THAN A 15 MINUTE NO DRUG PERIOD. D. THE NO DRUG PRE SESSION INITIATED WITH A 15 MINUTE NO DRUG PERIOD THEN CONTINUED TO A BASAL SESSION. E AND F. COCAINE-INDUCED REINSTATEMENT SESSIONS CONSISTED OF 60-MINUTE EXTINCTION PERIODS AND THEN 1 MINUTE OF THE DS PRESENTATION. IN THE DRUG DS CONDITION, THERE WAS NO CHANGE (E) WHILE IN THE NO DRUG DS CONDITION, THE LIGHT WAS SWITCHED TO THE ND DS FOR ONE MINUTE. (F). AFTERWARDS, NON-CONTINGENT INJECTIONS OF COCAINE AT INCREASING DOSE OCCURRED EVERY 30 MINUTES (0.2 MG/KG, 0.4 MG/KG, 0.8 MG/KG, AND 1.6 MG/KG) AND THE DS CONDITIONS WERE KEPT AS DESCRIBED FOR THE LAST MIN OF THE EXTINCTION PHASE..... 45

FIGURE 3: EXPERIMENTAL TIMELINE- CHAPTER 1- IN ALL EXPERIMENTS, RATS HAD AT LEAST 16 SESSIONS FOR SELF-ADMINISTRATION ACQUISITION. MANIPULATION 1 (NCI) OCCURRED FROM SESSIONS 17-32. MANIPULATION 2 (LONG ND) OCCURRED BETWEEN SESSIONS 21-28. MANIPULATION 3 (ND PERIOD PRE SESSION- ND PRE) OCCURRED FROM SESSIONS 18-36. MANIPULATION 4 (REINSTATEMENT) OCCURRED ON DAY 23..... 48

FIGURE 4: ACQUISITION: MANIPULATION 1- *MALES*: A. THERE WAS DISCRIMINATION BETWEEN THE ACTIVE AND INACTIVE HOLES IN THE DRUG PERIOD OVER THE ACQUISITION PERIOD. B. STABILITY IN COCAINE INJECTIONS TAKEN PER SESSION WAS ACHIEVED BY DAY 10. C. THERE WAS DISCRIMINATION BETWEEN ACTIVE AND INACTIVE HOLES IN THE FIRST DRUG PERIOD OVER THE ACQUISITION PERIOD. *FEMALES*: D. THERE WAS DISCRIMINATION BETWEEN THE ACTIVE AND INACTIVE HOLES IN THE DRUG PERIOD OVER THE ACQUISITION PERIOD. E. STABILITY IN COCAINE INJECTIONS TAKEN PER SESSION WAS ACHIEVED BY DAY 10. F. THERE WAS DISCRIMINATION BETWEEN ACTIVE AND INACTIVE HOLES IN THE FIRST DRUG PERIOD OVER THE ACQUISITION PERIOD. 52

FIGURE 5: ACQUISITION: MANIPULATION 1- EVOLUTION OF NO DRUG 1 RESPONDING IN HIGH AND LOW SEEKERS- *MALES*: DISCRIMINATION EVOLVED DIFFERENTLY IN ACTIVE AND INACTIVE HOLES IN HIGH AND LOW SEEKERS IN THE FIRST NO DRUG PERIOD. NOTABLY, WE OBSERVED THAT MALADAPTIVE SEEKING IN HIGH RATS STARTED TO OCCUR AT SESSION 7 WHILE THE LOW RATS DIMINISHED ACTIVE SEEKING OVER TIME AND EVENTUALLY NO LONGER DISCRIMINATED BETWEEN ACTIVE AND INACTIVE HOLES. 53

FIGURE 6: MANIPULATION 1: SELF-ADMINISTRATION BEHAVIOR IN HIGH AND LOW MALADAPTIVE SEEKERS – *MALES*: HIGH AND LOW SEEKERS DIFFERED IN A. TOTAL ACTIVE NO DRUG NOSE-POKES, HOWEVER, DID NOT SIGNIFICANTLY DIFFER IN B. TOTAL INJECTIONS OR C. INTER-INFUSION INTERVAL IN THE SESSIONS USED TO CHARACTERIZE THEM AS HIGH OR LOW SEEKERS. *** $p < 0.001$ *FEMALES*: HIGH AND LOW SEEKERS DIFFERED IN D. TOTAL ACTIVE NO DRUG NOSE-POKES, E. TOTAL INJECTIONS, AND F. INTER-INFUSION INTERVAL IN THE SESSIONS USED TO CHARACTERIZE THEM AS HIGH OR LOW SEEKERS. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ 55

FIGURE 7: MANIPULATION 1: NON-CONTINGENT INJECTION EFFECT (GLOBAL)- *MALES*: A. IN BOTH BASELINE AND NCI SESSIONS, THE FIRST 5 MINUTES WAS SIGNIFICANTLY DIFFERENT FROM THE LAST 10 MINUTES. THE FIRST 5 MINUTES OF BOTH BASELINE AND NCI SESSIONS DID NOT DIFFER. IN THE LAST 10 MINUTES, ACTIVE NOSE-POKES DECREASED SIGNIFICANTLY BETWEEN THE BASAL AND NCI CONDITIONS. **** $p < 0.0001$, *** $p < 0.001$, * $p < 0.05$ *FEMALES*: B. THE FIRST 5 MINUTES WAS SIGNIFICANTLY DIFFERENT FROM THE LAST 10 MINUTES OF THE BASELINE SESSION, BUT NOT IN THE NCI SESSION. THE FIRST 5 MINUTES OF BOTH BASELINE AND NCI SESSIONS DID NOT DIFFER. IN THE LAST 10 MINUTES, ACTIVE NOSE-POKES DECREASED SIGNIFICANTLY BETWEEN THE BASAL AND NCI CONDITIONS. **** $p < 0.0001$ 57

FIGURE 8: MANIPULATION 1: NON-CONTINGENT INJECTION EFFECT (HIGH/LOW)- MALES: A. IN HIGH MALADAPTIVE SEEKERS, THE FIRST FIVE MINUTES OF THE NO DRUG PERIOD IN THE BASAL AND NCI SESSIONS WERE EQUIVALENT, HOWEVER, THE ACTIVE NOSE-POKES IN THE LAST TEN MINUTES OF THE NO DRUG PERIOD BETWEEN THE BASAL AND NCI SESSIONS WERE SIGNIFICANTLY DIFFERENT SHOWING THAT THE NON-CONTINGENT INJECTION SUCCESSFULLY DECREASED MALADAPTIVE SEEKING PATTERNS. B. IN LOW MALADAPTIVE SEEKERS, THE FIRST 5 MINUTES DURING THE NO DRUG PERIOD IN THE BASAL AND NCI SESSIONS WERE ALSO EQUIVALENT. HOWEVER, UNLIKE THE HIGH RATS, THERE WAS NOT A SIGNIFICANT DIFFERENCE BETWEEN THE LAST TEN MINUTES OF THE BASAL AND NCI SESSIONS. NON-CONTINGENT INJECTIONS SIGNIFICANTLY AFFECT MALADAPTIVE SEEKING BEHAVIOR IN HIGH RATS WHILE THIS EFFECT IS NOT AS SIGNIFICANT IN LOW SEEKERS. **** $p < 0.0001$, * $p < 0.05$ FEMALES: C. IN HIGH MALADAPTIVE SEEKERS, THE FIRST FIVE MINUTES OF THE NO DRUG PERIOD IN THE BASAL AND NCI SESSIONS WERE EQUIVALENT; HOWEVER, THE ACTIVE NOSE-POKES IN THE LAST TEN MINUTES OF THE NO DRUG PERIOD BETWEEN THE BASAL AND NCI SESSIONS WERE SIGNIFICANTLY DIFFERENT SHOWING THAT THE NON-CONTINGENT INJECTION SUCCESSFULLY DECREASED MALADAPTIVE SEEKING PATTERNS. D. IN LOW MALADAPTIVE SEEKERS, THE FIRST 5 MINUTES DURING THE NO DRUG PERIOD IN THE BASAL AND NCI SESSIONS WERE ALSO EQUIVALENT. HOWEVER, UNLIKE THE HIGH RATS, THERE WAS NOT A SIGNIFICANT DIFFERENCE BETWEEN THE LAST TEN MINUTES OF THE BASAL AND NCI SESSIONS. NON-CONTINGENT INJECTIONS SIGNIFICANTLY AFFECT MALADAPTIVE SEEKING BEHAVIOR IN HIGH RATS WHILE THIS EFFECT IS NOT AS SIGNIFICANT IN LOW SEEKERS. **** $p < 0.0001$ 59

FIGURE 9: MANIPULATION 2: SELF-ADMINISTRATION BEHAVIOR IN HIGH AND LOW MALADAPTIVE SEEKERS- HIGH AND LOW SEEKERS DIFFERED IN A. TOTAL ACTIVE NO DRUG NOSE-POKES, BUT NOT IN B. TOTAL INJECTIONS OR C. INTER-INFUSION INTERVAL DURING THE SESSIONS USED TO CHARACTERIZE THEM AS HIGH OR LOW. ** $p < 0.01$ 61

FIGURE 10: MANIPULATION 2: LONG NO DRUG PERIOD SESSIONS (HIGH/LOW)- THE LONG NO DRUG PERIOD SEEKING PATTERNS DIFFERED IN HIGH AND LOW SEEKERS. HIGH SEEKERS HAD STRONG SEEKING PATTERNS IN THE BEGINNING OF THE LONG NO DRUG PERIOD, WHEREAS LOW SEEKERS HAD LOW SEEKING PATTERNS THROUGHOUT THE WHOLE LONG NO DRUG PERIOD, WITH NO SIGNIFICANT DIFFERENCE BETWEEN THE SIX 15-MINUTE BINS. 62

FIGURE 11: MANIPULATION 3: SELF-ADMINISTRATION BEHAVIOR IN HIGH AND LOW MALADAPTIVE SEEKERS- MALES: HIGH AND LOW SEEKERS DIFFERED IN A. TOTAL ACTIVE NOSE-POKES, B. TOTAL INJECTIONS, AND C. INTER-INFUSION INTERVAL DURING THE SESSIONS USED TO CHARACTERIZE THEM AS HIGH OR LOW. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$ FEMALES: HIGH AND LOW SEEKERS DIFFERED IN D. TOTAL ACTIVE NOSE-POKES, BUT NOT TOTAL INJECTIONS, NOR F. INTER-INFUSION INTERVAL DURING THE SESSIONS USED TO CHARACTERIZE THEM AS HIGH OR LOW. ** $p < 0.01$ 66

FIGURE 12: MANIPULATION 3: NO DRUG PRE (GLOBAL)- MALES: A. ACTIVE NOSE-POKES BETWEEN THE DRUG FREE NO DRUG PERIOD AND THE INTOXICATED NO DRUG PERIOD SIGNIFICANTLY DIFFERED AT THE POPULATION LEVEL ** $p < 0.01$ FEMALES: B. ACTIVE NOSE-POKES BETWEEN THE DRUG FREE NO DRUG PERIOD AND THE INTOXICATED NO DRUG PERIOD SIGNIFICANTLY DIFFERED AT THE POPULATION LEVEL * $p < 0.05$ 67

FIGURE 13: MANIPULATION 3: NO DRUG PRE (HIGH/LOW)- MALES: A. NO DRUG PRE ACTIVE NOSE-POKES DID NOT SIGNIFICANTLY DIFFER BETWEEN HIGH AND LOW SEEKERS. ADDITIONALLY, IN LOW SEEKERS, NO DRUG SEEKING WAS NOT STRONGLY INFLUENCED BY COCAINE- IT REMAINED EQUIVALENT BETWEEN DRUG FREE AND INTOXICATED STATES. HOWEVER, IN HIGH SEEKERS, THERE WAS A SIGNIFICANT INCREASE IN ACTIVE NOSE-POKES FROM A DRUG FREE NO DRUG PERIOD TO AN INTOXICATED NO DRUG STATE. *** $p < 0.001$ FEMALES: B. NO DRUG PRE ACTIVE NOSE-POKES DID NOT SIGNIFICANTLY DIFFER BETWEEN HIGH AND LOW SEEKERS. ADDITIONALLY, IN LOW SEEKERS, NO DRUG SEEKING WAS NOT STRONGLY INFLUENCED BY COCAINE- IT REMAINED EQUIVALENT BETWEEN DRUG FREE AND INTOXICATED STATES. HOWEVER, IN HIGH SEEKERS, THERE WAS A SIGNIFICANT INCREASE IN ACTIVE NOSE-POKES FROM A DRUG FREE NO DRUG PERIOD TO AN INTOXICATED NO DRUG STATE. *** $p < 0.001$ ** $p < 0.01$ 69

FIGURE 14: MANIPULATION 4: SELF-ADMINISTRATION BEHAVIOR IN HIGH AND LOW MALADAPTIVE SEEKERS- MALES: HIGH AND LOW SEEKERS DIFFERED IN A. TOTAL ACTIVE NOSE-POKES, B. TOTAL INJECTIONS, AND C. INTER-INFUSION INTERVAL DURING THE SESSIONS USED TO CHARACTERIZE THEM AS HIGH OR LOW. **** $p < 0.0001$, ** $p < 0.01$ FEMALES: HIGH AND LOW SEEKERS DID NOT SIGNIFICANTLY DIFFER IN D. TOTAL ACTIVE NOSE-POKES, E. TOTAL INJECTIONS, OR F. INTER-INFUSION INTERVAL DURING THE SESSIONS USED TO CHARACTERIZE THEM AS HIGH OR LOW..... 72

FIGURE 15: MANIPULATION 4: COCAINE-INDUCED REINSTATEMENT UNDER DRUG AND NO DRUG DS- MALES: A. BETWEEN THE DRUG AND NO DRUG DS GROUPS, EXTINCTION WAS EQUIVALENT. ND DS DECREASED COCAINE-INDUCED REINSTATEMENT WITH A

SPECIFIC EFFECT ON ACTIVE NOSE-POKING FOR THE TWO HIGHEST DOSES ADMINISTERED **** $p < 0.0001$, * $p < 0.05$ FEMALES: B. BETWEEN THE DRUG AND NO DRUG DS GROUPS, EXTINCTION WAS EQUIVALENT. GLOBAL RESPONDING IN COCAINE-INDUCED REINSTATEMENT WAS HIGHER IN THE DRUG DS GROUP THAN IT WAS FOR THE NO DRUG DS GROUP. 75

FIGURE 16: MANIPULATION 4: COCAINE-INDUCED REINSTATEMENT (ACTIVE AND INACTIVE HOLES) UNDER DRUG AND NO DRUG DS AS A FUNCTION OF VULNERABILITY- MALES: A. ACTIVE AND INACTIVE RESPONDING IN HIGH RATS UNDER BOTH D DS AND ND DS. DURING EXTINCTION AND AT 0.8/1.6 MG/KG/INFUSION, HIGH RATS IN BOTH DS CONDITIONS DISCRIMINATED BETWEEN THE ACTIVE AND INACTIVE HOLES. B. ACTIVE AND INACTIVE RESPONDING IN LOW RATS UNDER BOTH D DS AND ND DS. DURING EXTINCTION AND AT 1.6 MG/KG/INFUSION, RATS IN BOTH D DS AND ND DS CONDITIONS DISCRIMINATED BETWEEN THE ACTIVE AND INACTIVE HOLES. HOWEVER, AT 0.8 MG/KG/INFUSION, ONLY THE D DS GROUP HAD A SIGNIFICANT DIFFERENCE IN ACTIVE AND INACTIVE RESPONSES, DUE TO THE LOW LEVELS OF ACTIVE HOLE RESPONDING IN THE LOW ND DS GROUP. **** $p < 0.00001$, *** $p < 0.001$, * $p < 0.05$ AS COMPARED TO INACTIVE NOSE-POKES. 77

FIGURE 17: MANIPULATION 4: COCAINE-INDUCED REINSTATEMENT (ACTIVE HOLE) UNDER DRUG AND NO DRUG DS AS A FUNCTION OF VULNERABILITY (HIGH/LOW)- MALES: WHETHER IN D DS OR ND DS CONDITION, HIGH AND LOW SEEKERS DID NOT DIFFER IN EXTINCTION. HOWEVER, AT THE HIGHEST DOSE IN REINSTATEMENT, THE HIGH SEEKERS HAD SIGNIFICANTLY HIGHER ACTIVE NOSE-POKING ACTIVITY COMPARED TO THE LOW SEEKERS IN BOTH THE DRUG DS AND THE ND DS CONDITION. **** $p < 0.0001$, ** $p < 0.01$, AS COMPARED TO LOW SEEKERS. 78

FIGURE 18: METABOLISM OF COCAINE (FROM XIE ET AL., 1999)- COCAINE IS BROKEN DOWN INTO THREE METABOLITES: ECGONINE METHYL ESTER (EME), BENZOYLECGONINE (BZE), AND NORCOCAINE. EME IS A MAJOR METABOLITE FOUND IN HUMAN URINE AND IS PRODUCED BY HYDROLYSIS OF COCAINE IN THE BLOOD BY BCHE. THE SECOND METABOLITE FOUND IN HIGH AMOUNTS IN HUMAN URINE IS BZE, WHICH IS PRODUCED BY SPONTANEOUS HYDROLYSIS AND BY LIVER CARBOXYLESTERASE (BRZEZINSKI ET AL., 1994). LASTLY, NORCOCAINE IS PRODUCED BY DEMETHYLATION BY VARIOUS CYTOCHROME P450 (CYP) ENZYMES, HOWEVER AT MUCH LOWER CONCENTRATIONS COMPARED TO EME OR BZE. 84

FIGURE 19: SACRIFICE SESSION FOR BRAIN COCAINE AND METABOLITE QUANTIFICATION- ON THE DAY OF SACRIFICE, ALL RATS WERE PLACED IN THE SELF-ADMINISTRATION CHAMBER FOR FORTY MINUTES, RECEIVING 8 NON-CONTINGENT INJECTIONS EVERY FIVE MINUTES. IMMEDIATELY AFTERWARDS, THEY WERE SACRIFICED, AND THEIR BRAINS WERE COLLECTED AND SNAP FROZEN. PUNCHING OF THE STRUCTURES OF INTEREST WAS PERFORMED, THEN THE COCAINE AND METABOLITE CONCENTRATION WAS ANALYZED THROUGH LC-MS/MS. 86

FIGURE 20: BRAIN COCAINE/METABOLITE EXPERIMENT: SELF-ADMINISTRATION BEHAVIOR IN HIGH AND LOW MALADAPTIVE SEEKERS- HIGH AND LOW SEEKERS DIFFERED IN A. TOTAL ACTIVE NOSE-POKES, B. TOTAL INJECTIONS, AND C. INTER-INFUSION INTERVAL DURING THE SESSIONS USED TO CHARACTERIZE THEM AS HIGH OR LOW. **** $p < 0.0001$, * $p < 0.05$ 91

FIGURE 21: COCAINE AND METABOLITE CONCENTRATION IN THE BRAIN REGIONS COLLECTED AFTER NON CONTINGENT COCAINE INFUSIONS- ALL RATS WERE ADMINISTERED WITH 8 NON CONTINGENT COCAINE IV INFUSIONS, ONE EVERY 5 MIN, OVER 40 MIN, AND SACRIFICED IMMEDIATELY AFTER FOR BRAIN COLLECTION. A. COCAINE, B. EME, C. BZE, AND D. NORCOCAINE CONCENTRATION FOUND IN EACH BRAIN REGION. 92

FIGURE 22: RELATIONSHIP BETWEEN VTA [COCAINE] AND BODY WEIGHT- THERE WAS A CORRELATION BETWEEN THE CONCENTRATION OF COCAINE IN THE VTA AND THE BODY WEIGHT OF THE ANIMAL AT SACRIFICE. 96

FIGURE 23: RELATIONSHIP BETWEEN PL COCAINE CONCENTRATION AND SELF-ADMINISTRATION BEHAVIORS- A. RATS WITH HIGH COCAINE CONCENTRATION FOUND IN THE PL AFTER EQUAL NON-CONTINGENT COCAINE HAD SLOWER RATES OF INTAKE IN THE SELF-ADMINISTRATION SESSION THE DAY BEFORE SACRIFICE THAN THOSE WITH LOW COCAINE CONCENTRATION. B. THERE WAS A POSITIVE CORRELATION BETWEEN THE INTER-INFUSION INTERVAL IN THE SESSION THE DAY BEFORE SACRIFICE AND THE CONCENTRATION OF COCAINE IN THE PL. C. RATS WITH HIGH COCAINE CONCENTRATION FOUND IN THE PL HAD SIGNIFICANTLY HIGHER INTAKE IN THE SESSION THE DAY BEFORE SACRIFICE THAN THOSE WITH LOW COCAINE CONCENTRATION. D. THERE WAS A NEGATIVE CORRELATION BETWEEN THE NUMBER OF COCAINE INJECTIONS IN THE SESSION THE DAY BEFORE SACRIFICE AND THE CONCENTRATION OF COCAINE IN THE PL. E. RATS WITH HIGH AND LOW COCAINE CONCENTRATION FOUND IN THE PL HAD NO DIFFERENCE IN ACTIVE NOSE-POKES DURING THE FIRST NO DRUG PERIOD IN THE SESSION THE DAY BEFORE SACRIFICE. F. UNLIKE INTER-INFUSION INTERVAL AND

INJECTIONS, THERE WAS NO RELATIONSHIP BETWEEN PL COCAINE CONCENTRATION AND ACTIVE NOSE-POKES IN THE FIRST NO DRUG PERIOD IN THE SESSION THE DAY BEFORE SACRIFICE BETWEEN RATS WITH HIGH AND LOW COCAINE IN THE PL..... 99

FIGURE 24: EVOLUTION OF NO DRUG RESPONDING IN RATS WITH HIGH AND LOW COCAINE IN THE PL- A. RATS WITH HIGH PL COCAINE DID NOT HAVE AN EVOLUTION OF RESPONDING IN THE NO DRUG PERIOD BETWEEN THE FIRST 6 SESSIONS AND THE SUBSEQUENT 10 SESSIONS, WHEREAS THE RATS WITH LOW PL COCAINE HAD A NEARLY SIGNIFICANT ($p=0.0516$) DIFFERENCE IN THE ACTIVE NOSE-POKES BETWEEN THE TWO TIME POINTS. 100

FIGURE 25: COMPARISON OF BRAIN PL COCAINE CONCENTRATION AS A FUNCTION OF MALADAPTIVE SEEKING GROUP- HIGH AND LOW MALADAPTIVE SEEKERS DID NOT DIFFER IN PL COCAINE CONCENTRATION ($p=0.1879$). 101

FIGURE 26: RELATIONSHIP OF BChE ACTIVITY AND BODY WEIGHT- A. THERE WAS A TENDENCY IN THE NAÏVE CONDITION THAT THE ACTIVITY OF BChE WAS RELATED TO THE BODY WEIGHT OF THE ANIMAL. B. AT SACRIFICE, THIS RELATIONSHIP WAS NOT MAINTAINED. 106

FIGURE 27: BChE ACTIVITY BEFORE AND AFTER COCAINE SELF-ADMINISTRATION- A. THERE WAS A SIGNIFICANT DECREASE IN BChE ACTIVITY AFTER 25 DAYS OF INTRAVENOUS COCAINE SELF-ADMINISTRATION. $**p<0.01$ 107

FIGURE 28: RELATIONSHIP OF BChE ACTIVITY AND INTER-INFUSION INTERVAL- A. IN THE NAÏVE CONDITION, BChE ACTIVITY WAS NOT ASSOCIATED WITH THE AVERAGE RATE OF INTAKE IN SESSIONS 2 AND 3. B. AT SACRIFICE, HIGHER BChE ACTIVITY WAS ASSOCIATED WITH A LONGER AVERAGE INTER-INFUSION INTERVAL IN THE TWO SESSIONS BEFORE SACRIFICE..... 108

FIGURE 29: RELATIONSHIP OF BChE ACTIVITY AND MALADAPTIVE SEEKING PATTERNS- BChE ACTIVITY AT SACRIFICE WAS NOT RELATED TO THE NUMBER OF AVERAGE ACTIVE NOSE-POKES IN THE FIRST NO DRUG PERIOD IN THE TWO SESSIONS BEFORE SACRIFICE. 108

FIGURE 30: RELATIONSHIP OF BChE ACTIVITY AND MALADAPTIVE SEEKING GROUP- BChE ACTIVITY AT SACRIFICE WAS NOT SIGNIFICANTLY DIFFERENT BETWEEN HIGH AND LOW SEEKERS ($p=0.51$)..... 109

FIGURE 31: FIBER PHOTOMETRY RECORDINGS- A. IN RECORDED SELF-ADMINISTRATION SESSIONS, FIBER PHOTOMETRY RECORDINGS OCCURRED FOR 4 MINUTES SURROUNDING THE SWITCH FROM THE FIRST DRUG TO THE FIRST NO DRUG PERIOD. B. IN 6/11 OF THE RECORDINGS USED FOR ND PRE ANALYSIS, THE SWITCH TO THE ND PRE OCCURRED FROM DARKNESS. C. IN THE REMAINING SESSIONS, THE SWITCH TO THE ND PRE OCCURRED AFTER A SHORT (10 SECOND) PRESENTATION OF THE DRUG DS. THE SECOND RECORDING (SWITCH FROM THE DRUG PERIOD TO THE INTOXICATED NO DRUG PERIOD) REMAIN UNCHANGED IN THE TWO SESSIONS. D. IN THE RECORDED NCI BASELINE SESSION, THE FIBER PHOTOMETRY RECORDING OCCURRED FOR 16 MINUTES: FROM THE LAST MINUTE OF THE DRUG PERIOD TO THE END OF THE FIRST NO DRUG PERIOD. E. THE NCI RECORDING SESSION WAS IDENTICAL TO THE NCI BASELINE SESSION, BUT WITH TWO NON-CONTINGENT INJECTIONS IN THE NO DRUG PERIOD. 120

FIGURE 32: COUPLING OF FIBER PHOTOMETRY WITH SELF-ADMINISTRATION- DURING THE RECORDING SESSIONS, THE RATS WERE CONNECTED TO A DUAL PHARMACOLOGY/OPTIC FIBER-MOTORIZED COMMUTATOR WHICH ENSURED THAT THE PATCH CORD AND THE LINE CONTAINING THE COCAINE SOLUTION ROTATED SMOOTHLY AND IN PARALLEL TO PREVENT TANGLING OF THE TWO LINES. FIBER PHOTOMETRY RECORDINGS WERE INITIATION AND STOPPED BY A TTL PULSE GENERATED BY THE IMETRONIC BEHAVIORAL SYSTEM. CREATED WITH BIORENDER.COM. 121

FIGURE 33: HISTOLOGY- A. AN EXAMPLE HISTOLOGICAL SLICE SHOWING FIBER PLACEMENT AND VIRAL GCAMP EXPRESSION. B. LOCATIONS OF THE FIBER TIP IN ALL RATS USED FOR ANALYSIS. 122

FIGURE 34: EXPERIMENTAL TIMELINE: CHAPTER 3- IN ALL EXPERIMENTS, RATS HAD AT LEAST 16 SESSIONS FOR ACQUISITION. BASAL SELF-ADMINISTRATION RECORDINGS HAPPENED FROM SESSION 17-46 WHILE RECORDED ND PRE SESSIONS OCCURRED FROM SESSION 28-36. NCI BASELINE SESSIONS OCCURRED FROM SESSION 38-45 WHILE NCI SESSIONS OCCURRED FROM SESSION 34-41. 123

FIGURE 35: PL RECRUITMENT IN RESPONSE TO PRESENTATION OF THE NO DRUG DS AND MALADAPTIVE SEEKING PATTERNS- A. THERE WAS A CORRELATION BETWEEN THE AVERAGE Z-SCORE (3s) AFTER PRESENTATION OF THE ND DS AND THE LATENCY TO THE FIRST ACTIVE NOSE-POKE IN THE NO DRUG PERIOD. B. THERE WAS A TENDENCY THAT THE AVERAGE Z-SCORE (3s) AFTER PRESENTATION OF THE ND DS WAS ALSO CORRELATED TO THE TOTAL NUMBER OF ACTIVE NOSE-POKES IN THE NO DRUG PERIOD, HOWEVER IT WAS NOT SIGNIFICANT ($p=0.0874$). C. REMOVING AN EXTREME RAT THAT WAS NOSE-POKING BETWEEN 900-1300 TIMES IN THE 15 MINUTE NO DRUG PERIOD LED TO SIGNIFICANCE OF THE CORRELATION BETWEEN THE AVERAGE PL RECRUITMENT (3s) IN RESPONSE TO THE ND DS AND THE NUMBER OF ACTIVE NOSE-POKES DURING THE SIGNALLED NO DRUG PERIOD. 134

FIGURE 36: PL RECRUITMENT IN RESPONSE RATE OF INTAKE AND NUMBER OF INJECTIONS- A. THERE WAS A TENDENCY, HOWEVER NO CORRELATION BETWEEN THE AVERAGE Z-SCORE (3s) AFTER PRESENTATION OF THE ND DS AND THE NUMBER OF COCAINE INJECTIONS OBTAINED IN THE DRUG PERIOD JUST PRIOR TO THE RECORDED ONSET OF THE ND PERIOD. B. THERE WAS A TENDENCY, HOWEVER NO CORRELATION BETWEEN THE AVERAGE Z-SCORE (3s) AFTER PRESENTATION OF THE ND DS AND THE RATE OF COCAINE INTAKE IN THE DRUG PERIOD JUST PRIOR TO THE RECORDED ONSET OF THE ND PERIOD..... 135

FIGURE 37: PL RECRUITMENT IN RESPONSE RATE OF INTAKE AND NUMBER OF INJECTIONS- THERE WAS NO CORRELATION BETWEEN THE AVERAGE Z-SCORE (3s) AFTER PRESENTATION OF THE ND DS AND THE LATENCY TO THE LAST INJECTION BEFORE THE RECORDED ONSET OF THE ND PERIOD..... 136

FIGURE 38: PEAK FREQUENCY/AMPLITUDE ANALYSIS FOR ND PRE SESSIONS- A. PEAK FREQUENCY AND B. PEAK AMPLITUDE DID NOT SIGNIFICANTLY DIFFER IN THE BASELINE PERIOD BEFORE THE SWITCH TO THE ND PRE PERIOD. 137

FIGURE 39: PL RECRUITMENT AND BEHAVIOR IN DRUG FREE ND PERIOD VS INTOXICATED ND PERIOD- A. AT THE POPULATION LEVEL, THE RECRUITMENT OF THE PL BY THE ND DS DID NOT DIFFER BETWEEN THE DRUG FREE AND THE INTOXICATED STATE ($p=0.9282$, NS) DESPITE THE FACT THAT B. SEEKING BEHAVIOR DURING NO DRUG DIFFERED SIGNIFICANTLY ACROSS ALL ANIMALS BETWEEN THE NO DRUG PRE TO THE INTOXICATED NO DRUG PERIOD. * $p<0.05$ 138

FIGURE 40: BEHAVIOR IN DRUG FREE ND PERIOD VS INTOXICATED ND PERIOD AS A FUNCTION OF VULNERABILITY- A. HIGH AND LOW GROUPS DID NOT DIFFER IN ACTIVE NOSE-POKES IN THE ND PRE PERIODS, WHILE SIGNIFICANTLY DIFFERING IN THE INTOXICATED ND PERIOD. ADDITIONALLY, THE LOW GROUP DID NOT SIGNIFICANTLY DIFFER IN ACTIVE NOSE-POKES BETWEEN THE DRUG FREE AND THE INTOXICATED ND PERIODS WHILE THE HIGH RATS INCREASED ACTIVE NOSE-POKES SIGNIFICANTLY IN THE INTOXICATED NO DRUG PERIOD COMPARED TO THE DRUG FREE ND PERIOD. HOWEVER, THE HIGH AND LOW GROUPS DID NOT DIFFER IN B. INJECTIONS OR C. INTER-INFUSION INTERVAL IN THE DRUG PERIOD BETWEEN THE TWO NO DRUG PERIODS. *** $p<0.001$ 139

FIGURE 41: PL RECRUITMENT IN DRUG FREE ND PERIOD VS INTOXICATED ND PERIOD AS A FUNCTION OF VULNERABILITY- A. THE TWO GROUPS DID NOT DIFFER SIGNIFICANTLY OVER THE TWO CONDITIONS [N=11, GROUP EFFECT: $F(1, 9)=2.0369$, $p=0.18729$] NOR AS A FUNCTION OF THE TESTED CONDITION (ND PRE VS INTOXICATED ND1) [MALADAPTIVE GROUP X CONDITION EFFECT: $F(1, 9)=1.6468$, $p=0.23146$]. THOUGH IT DID NOT REACH SIGNIFICANCE (PROBABLY DUE TO LIMITED NUMBER OF RATS) THERE WAS A TENDENCY FOR THE LOW ANIMALS TO HAVE HIGHER RECRUITMENT OF THE PL COMPARED TO THE HIGH ANIMALS WHEN PRESENTED WITH THE ND DS IN THE INTOXICATED STATE. THE HIGH ANIMALS SEEMED TO RECRUIT THE PL MORE IN A DRUG FREE STATE COMPARED TO AN INTOXICATED STATE, WHILE THE LOW ANIMALS SEEMED TO RECRUIT THE PL MORE IN AN INTOXICATED STATE AS COMPARED TO A DRUG FREE STATE. 140

FIGURE 42: PL RECRUITMENT IN DRUG FREE ND PERIOD VS INTOXICATED ND PERIOD RELATED TO MALADAPTIVE SEEKING- A. IN THE DRUG FREE ND PERIOD, RECRUITMENT OF THE PL WAS NOT CORRELATED TO THE LATENCY TO THE FIRST ACTIVE NOSE-POKE NOR C. THE TOTAL ACTIVE NOSE-POKES OVER THE NO DRUG PERIOD. B. IN THE INTOXICATED ND PERIOD, THE RECRUITMENT OF THE PL WAS NOT CORRELATED TO THE LATENCY TO THE FIRST ACTIVE NOSE-POKE. D. THERE WAS A TREND THAT THE RECRUITMENT OF THE PL WAS INVERSELY RELATED TO THE NUMBER OF ACTIVE NOSE-POKES IN THE NO DRUG PERIOD. 141

FIGURE 43: NCI BASELINE- A. RATS HAD SIGNIFICANTLY DIFFERENT BEHAVIOR IN THE FIRST 5 MINUTES COMPARED TO THE LAST 10 MINUTES OF THE NO DRUG PERIOD. B. THERE WAS A TREND THAT THE PEAK AMPLITUDE INCREASED FROM THE FIRST FIVE MINUTES TO THE LAST TEN MINUTES OF THE NO DRUG PERIOD, HOWEVER IT DID NOT REACH SIGNIFICANCE. C. THE PEAK FREQUENCY DID NOT SIGNIFICANTLY DIFFER BETWEEN THE FIRST FIVE MINUTES AND LAST TEN MINUTES OF THE NO DRUG PERIOD. 142

FIGURE 44: EFFECT OF NCI ON NO DRUG SEEKING AND PL ACTIVITY – A. THOUGH THERE WAS NO EFFECT OF CONDITION, TIME, NOR CONDITION X TIME, THERE WAS A VISUALLY-IDENTIFIED REDUCTION IN ACTIVE NO DRUG NOSE-POKES WHEN COMPARING THE AVERAGE ACTIVE NOSE-POKES IN THE LAST TEN MINUTES OF THE PREVIOUS NO DRUG PERIODS TO THE NUMBER OF ACTIVE NOSE-POKES IN THE LAST TEN MINUTES OF THE NCI NO DRUG PERIOD. B. RATS DID NOT HAVE SIGNIFICANTLY DIFFERENT BEHAVIOR IN THE FIRST 5 MINUTES COMPARED TO THE LAST 10 MINUTES OF THE NCI NO DRUG PERIOD. C. THERE WAS DECREASE IN THE PEAK AMPLITUDE FROM THE FIRST FIVE MINUTES TO THE LAST TEN MINUTES OF THE NCI NO DRUG PERIOD. D. THE PEAK FREQUENCY DID NOT SIGNIFICANTLY DIFFER BETWEEN THE FIRST FIVE MINUTES AND LAST TEN MINUTES OF THE NCI NO DRUG PERIOD..... 144

INDEX OF TABLES

TABLE 1: DIAGNOSTIC CRITERIA FOR SUD FOUND IN THE DSM-5- TABLE INCLUDING THE NUMBER OF CRITERIA FOR MILD, MODERATE, AND SEVERE SUBSTANCE USE DISORDERS. 21

TABLE 2 : CORRELATIONS OF [COCAINE] AND [METABOLITES] IN EACH BRAIN REGION AFTER NON CONTINGENT COCAINE INFUSIONS- ALL RATS WERE ADMINISTERED WITH 8 NON CONTINGENT COCAINE IV INFUSIONS, ONE EVERY 5 MIN, OVER 40 MIN, AND SACRIFICED IMMEDIATELY AFTER FOR BRAIN COLLECTION. TABLE CONTAINING CORRELATIONS BETWEEN THE COCAINE CONCENTRATION AND EME, BZE, AND NORCOCAINE LEVELS. ALL STRUCTURES HAD CORRELATIONS BETWEEN [COCAINE] AND [EME] / [COCAINE] AND [NORCOCAINE]. ALL STRUCTURES EXCEPT ACC AND NACSH HAD CORRELATIONS BETWEEN [COCAINE] AND [BZE]..... 94

TABLE 3: CORRELATIONS BETWEEN [COCAINE] AND BODY WEIGHT- COCAINE CONCENTRATION WAS NOT CORRELATED TO BODY WEIGHT IN ANY STRUCTURE APART FROM THE VTA. 95

TABLE 4: CORRELATIONS BETWEEN [BRAIN COCAINE] AND COCAINE INTAKE- ALL RATS WERE ADMINISTERED WITH 8 NON CONTINGENT COCAINE IV INFUSIONS, ONE EVERY 5 MIN, OVER 40 MIN, AND SACRIFICED IMMEDIATELY AFTER FOR BRAIN COLLECTION. BRAIN COCAINE CONCENTRATION WERE RELATED TO SELF-ADMINISTRATION BEHAVIOR PREVIOUSLY EXPRESSED. CORRELATIONS WERE OBSERVED IN THE IL, PL, DLS, DMS, NACC, AND HB BETWEEN [COCAINE] AND BOTH INTER-INFUSION INTERVAL AND TOTAL INJECTIONS OVER THE WHOLE SELF-ADMINISTRATION SESSION BEFORE SACRIFICE. IN THE BLA, THERE WAS A CORRELATION BETWEEN [COCAINE] AND TOTAL INJECTIONS, HOWEVER THE CORRELATION WAS NOT SIGNIFICANT FOR III. IN THE OF, ACC, NACSH, AND VTA, [COCAINE] WAS NOT CORRELATED WITH NEITHER INTER-INFUSION INTERVAL NOR TOTAL INJECTIONS. 97

TABLE 5 : CORRELATION OF [EME]/[COCAINE] AFTER NON CONTINGENT COCAINE INFUSIONS AND SELF-ADMINISTRATION BEHAVIOR- TABLE CONTAINING CORRELATIONS BETWEEN THE [EME]/[COCAINE] RATIO AND AVERAGE INTER-INFUSION INTERVAL, TOTAL INJECTIONS, AND NO DRUG ACTIVE NOSE-POKES (ND1) IN THE SESSION BEFORE SACRIFICE..... 102

TABLE 6 : CORRELATION OF [BZE]/[COCAINE] AFTER NON CONTINGENT COCAINE INFUSIONS AND SELF-ADMINISTRATION BEHAVIOR- TABLE CONTAINING CORRELATIONS BETWEEN THE [BZE]/[COCAINE] RATIO AND AVERAGE INTER-INFUSION INTERVAL, TOTAL INJECTIONS, AND NO DRUG ACTIVE NOSE-POKES (ND1) IN THE SESSION BEFORE SACRIFICE..... 103

TABLE 7 : CORRELATION OF [NORCOCAINE]/[COCAINE] AFTER NON CONTINGENT COCAINE INFUSIONS AND SELF-ADMINISTRATION BEHAVIOR- TABLE CONTAINING CORRELATIONS BETWEEN THE [NORCOCAINE]/[COCAINE] RATIO AND AVERAGE INTER-INFUSION INTERVAL, TOTAL INJECTIONS, AND NO DRUG ACTIVE NOSE-POKES (ND1) IN THE SESSION BEFORE SACRIFICE. 104

LIST OF ABBREVIATIONS

ACC: Anterior Cingulate Cortex

BZE: Benzoyllecgonine

BChE: Butyrylcholinesterase

BLA: Basolateral Amygdala

CUD: Cocaine use disorder

D: Drug

DLS: Dorsolateral Striatum

DMS: Dorsomedial Striatum

DS: Discriminative stimulus

EME: Ecgonine Methyl Ester

FP: Fiber photometry

HB: Habenula

IL: Infralimbic Cortex

IV: Intravenous

LC-MS/MS: Liquid chromatography-tandem mass spectrometry

NacC: Nucleus Accumbens Core

NacSh: Nucleus Accumbens Shell

ND: No drug

OF: Orbitofrontal cortex

PL: Prelimbic cortex

SA: Self-administration

SEM: Standard error of the mean

VTA: Ventral Tegmental Area

PREFACE

Drug use and Substance Use Disorder (SUD) is widespread throughout the world- with consequences impacting public health, the criminal justice system, and importantly, impacting millions of people on the individual level. Cocaine is the second-most used illicit drug in the world behind cannabis (Karila et al., 2014) and rates of cocaine use have been steadily increasing in Europe (United Nations Office on Drugs and Crime, 2010). This is parallel with previous reports claiming that there is an increased production of cocaine and increased level of cocaine seizures, remarkably with increased purity (European Monitoring Centre for Drugs and Drug Addiction., 2021). Additionally, within Europe, first-time entries to treatment facilities for cocaine use problems have increased in seventeen countries.

However, despite the apparent increase in individuals suffering from Cocaine Use Disorder (CUD), there is a lack of effective treatment options. To date, the most effective treatments consist of psychosocial treatments (contingency management and/or cognitive behavioral therapy), and even then, they provide limited efficacy. There are pharmacological interventions that are being explored, however, to date there are no approved medications for the treatment of cocaine use disorder (Kampman, 2019).

This lack of effective treatment options highlights the limited predictive validity of preclinical models of addiction and the still limited knowledge on the psychobiological mechanisms supporting CUD. Animal models for the study of addictive mechanisms have the potential to address mechanistic questions about addiction because they permit longitudinal studies, allow identification of preexisting traits that predict progression into addiction, and allow studying drug-induced adaptations throughout the progression from early controlled stages of use through late addiction-like use in manners not possible in humans. However, to do it in a reliable way, these preclinical studies need to incorporate key features of clinical addiction such as maladaptive drug seeking: a main clinical challenge and behavioral handicap of cocaine addiction. They also need to take in account the fact that some, but not all, users progress from controlled to addictive use (Deroche-Gamonet, 2020, in press; Deroche-Gamonet & Piazza, 2014).

This doctoral dissertation focuses on psychobiological and neurobiological correlates of individual vulnerability to develop maladaptive cocaine seeking in a rat model of cocaine addiction-like behavior.

This dissertation is divided into:

An introduction starting by a discussion about the burdens of problematic cocaine use, then continues to a literature review of previous research that serves as the groundwork for the current dissertation, and ending with the hypothesis and specific objectives of the PhD work.

Three experimental chapters respectively devoted to uncovering and dissecting psychobiological mechanisms, pharmacokinetic influences, and neurobiological correlates of maladaptive seeking behavior.

Lastly, the dissertation's conclusion discusses the general findings more globally, re-emphasizing the importance of focusing on maladaptive seeking- a major behavioral handicap- in those who suffer from problematic cocaine use.

As a conclusion, we touch on the perspectives of future research that can be conducted that are grounded in the findings from this dissertation.

INTRODUCTION

Cocaine Use Disorder (CUD) is a type of Substance Use Disorder (SUD) related to the use of the psychostimulant cocaine, with the most severe form of CUD occurring in about 10 to 20% of cocaine users. Its defining criteria include problems at limiting drug use and drug seeking despite harmful consequences on physical, mental, and social health.

There are opposing views on the motives of this maladaptive drug seeking and taking, the relative role of drug vs other factors in sustaining use, and even on whether or not addiction should be classified as a disease (Heather et al., 2018). Whether it is a disease or not is a matter of perspective, but no one can doubt that a substantial number of drugs users ask for support because their drug taking becomes maladapted to their external environment, even if it might be adapted to their internal one. Maladaptive drug seeking (seeking for the drug while it is unbeneficial or risky) constitutes a major pathognomonic handicap and clinical challenge of drug addiction. The maladaptive nature of drug seeking may vary depending on societies and species, but it might be an operational key to animal modeling and translational relevance.

From a psychobiological point of view, maladaptive cocaine seeking involves increased response to drug-related incentives and a poor capacity to refrain from seeking (Hyman, 2012). Research has intensively explored the psycho- and neuro-biological mechanisms that drive excessive response to drug-related incentives, but only partially those involved in adaptive inhibitory control over drug seeking, and even less the balance between the two. Because empowering adaptive inhibition over cocaine craving in addicted users is a major clinical challenge, a better understanding of this balance is critical to develop novel therapeutic approaches. No pharmacological treatments with documented efficacy for cocaine addiction are currently available, however evidence-based psychological treatments targeting inhibitory control exist (contingency management and cognitive behavioral therapy). However, they are infrequently used in healthcare due to the limited knowledge surrounding their mechanisms of action as well as the specific demographics of the population that would most benefit from them.

Mechanisms of addiction are not easily traceable in human subjects due to methodological or ethical limitations. Animal models have the potential to address mechanistic questions about addiction, especially if they incorporate key clinical features of addiction, such as the distinction between controlled use and maladaptive use. They also need to take into consideration that some, but not all, users progress from controlled to addictive use (Deroche-Gamonet, 2020, in press; Deroche-Gamonet & Piazza, 2014). A particular strength of animal models is that they

permit longitudinal studies, allow identification of preexisting traits that predict progression into addiction, and allow studying drug-induced adaptations throughout the progression from early controlled stages of use through late addiction-like use, in a manner not possible in humans.

A model was developed for the study of cocaine addiction in the rat that incorporated key clinical features of addiction (Deroche-Gamonet et al., 2004). The daily training protocol alternates Drug (D) and No Drug (ND) periods signaled by specific discriminative stimuli (DS). Evaluation of addiction-like phenotypes is built from the clinical definition of addiction and consists of three addiction-like criteria, which incorporate one motivational and two maladaptive dimensions: 1. High motivation for the drug (evaluated through a progressive ratio test), 2. Difficulty to refrain from drug seeking in the signaled periods of drug unavailability (evaluated on a daily basis through the ND periods), 3. Maintenance of self-administration despite negative consequences (evaluated through punishment of seeking and taking by mild electric footshocks). Extreme responses in the three addiction-like behaviors occurs in about 15-20% of rats (3-crit) after protracted cocaine intravenous self-administration (SA) despite having drug use that is equivalent to the non-addicted group (0-crit) (Belin et al., 2009; Deroche-Gamonet et al., 2004). Vulnerability to develop a 3-crit addiction-like phenotype after protracted cocaine self-administration is preceded by an early high cocaine-induced incentive effects as measured through cocaine-induced reinstatement (Belin et al., 2009). Importantly, it is also preceded by the emergence of an early maladaptive seeking during the ND periods, especially selectively in the end two thirds of the ND period (in minutes 5-15 of the 15-minute no drug period) (Kasanez et al., 2010) and a fast pattern of use (Belin et al., 2009).

My thesis focuses on studying the psychobiological factors underlying this early default in adaptively inhibiting cocaine seeking. Combining behavioral methods, analytical chemistry and calcium imaging, I studied correlates of this early maladaptive seeking behavior in relation to psychopharmacology (objective 1), cocaine distribution/pharmacokinetic (objective 2), and neurobiology (objective 3).

In this introduction, I will first go through an overview of the scientific context and then present the general hypothesis and the three specific objectives of my PhD work.

SCIENTIFIC CONTEXT

COCAINE USE DISORDERS (CUD)

Economic and Social Burden of Problematic Cocaine Use

As mentioned in the preface, cocaine is the second-most used illicit drug in the world, behind cannabis (Karila et al., 2014). Problematic cocaine use results in a high cost to society. Economically speaking, there is a large financial burden of problematic cocaine use on the criminal justice system both due to crack cocaine being one of the two drugs that most frequently lead to crime (National Drug Intelligence Center, 2010), as well as cocaine serving as the primary drug type for those incarcerated for drug-related offenses in federal prisons (Taxy & Samuels, 2015).

Socially speaking, there is a burden of problematic drug use on the foster care system. From 2012 to 2017, there was an increase in children entering the foster care system with a stark increase of foster care entries that were attributable to parental drug use (Meinhofer & Angleró-Díaz, 2019). The percentage of these entries which are specifically due to crack cocaine/powdered cocaine use is not indicated; therefore, it is difficult to draw any precise conclusions on the effect of cocaine alone. Other social impacts of problematic cocaine use include but are not limited to homelessness and unemployment, leading to dependence on welfare (Daley, 2013).

Problematic cocaine use also leads to disruptions to family and social support. The instability that accompanies and emotional burden on the individual with the problematic use as well as the individuals surrounding them is traumatic and serves as an influential factor in the search for effective treatments (Daley, 2013).

Diagnostic Criteria of Problematic Cocaine Use

Worldwide, substance use disorders (SUD) are classified according to criteria defined by the American Psychological Association (APA) through the Diagnostic and Statistical Manual of Mental Disorders (DSM) (American Psychiatric Association, 2013) or by the World Health Organization (WHO) through the International Statistical Classification of Diseases and Related Health Problems (ICD) (World Health Organization, 1993). The clinical view of substance use disorders has changed drastically over the last 30 to 40 years. From the DSM-III (American Psychiatric Association & American Psychiatric Association, 1987) to the DSM-5, the clinical definition progressively switched from a mere drug use maintained and amplified by pharmacological factors (tolerance and

withdrawal symptoms), to a difficulty to control drug use and drug seeking, maintained despite negative consequences, and at the expense of other adaptive activities. Starting with the DSM-5, levels of severity are now defined by the number of symptoms expressed among 11 and go from no disorder to mild, moderate, or severe SUD (**Table 1**). Severe SUD occurs in only about 10 to 20% of cocaine users.

DSM-5 criteria for substance use disorder (SUD)	
1.	The stimulant is often taken in larger amounts or over a longer period than was intended
2.	There is a persistent desire or unsuccessful efforts to cut down or control stimulant use
3.	A great deal of time is spent in activities necessary to obtain the stimulant, use the stimulant, or recover from its effects
4.	Craving, or a strong desire or urge to use the stimulant
5.	Recurrent stimulant use resulting in a failure to fulfill major role obligations at work, school, or home
6.	Continued stimulant use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of the stimulant
7.	Important social, occupational, or recreational activities are given up or reduced because of stimulant use
8.	Recurrent stimulant use in situations in which it is physically hazardous
9.	Continued use despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the stimulant
10.	Tolerance
11.	Withdrawal
Severity	
Mild: Presence of 2-3 symptoms	
Moderate: Presence of 4-5 symptoms	
Severe: presence of 6+ symptoms	

TABLE 1: Diagnostic criteria for SUD found in the DSM-5- Table including the number of criteria for mild, moderate, and severe substance use disorders.

Maladaptive Drug Use, the Addiction Cycle, and Craving

In individuals with problematic cocaine use, their drug consumption becomes maladaptive, meaning that it starts to occur in inappropriate times and despite negative financial, health, and social consequences. This is thought to be highly dependent on impairments in both response inhibition and salience attribution- both being associated with prefrontal cortex dysfunction (Ceceli et al., 2022; Ersche et al., 2020; Goldstein & Volkow, 2011). Notably, these impairments contribute to the addiction cycle: repeated alternation of phases of craving, intoxication, and withdrawal (Koob & Volkow, 2010). This cycle, and specifically the craving phase of the addiction cycle, is highly relevant as it is something that is focused on in the clinic (Tiffany & Wray, 2012).

Current Treatments for Problematic Cocaine Use

Treatments in the clinic focus on the aforementioned craving phase of the addiction cycle, however currently, treatment options for individuals with cocaine use disorder are limited (Kampman, 2019).

- **Pharmacotherapies**

Pharmacological treatments are aimed to reduce/eliminate the occurrence of craving in individuals receiving treatment. To date, there are no approved medications that are used for treatment of CUD. However, there are some families of medications that show promising potential for the treatment of CUD. The first and most successful trials have occurred with dopamine agonists, such as long acting dextroamphetamine/amphetamine salts and modafinil (Grabowski et al., 2001, 2004; Kampman et al., 2015; Levin et al., 2015; Mooney et al., 2009; Nuijten et al., 2016). They have shown promise in both human and animal studies (Allain et al., 2021). Additional prospective medications also include the GABA agonist/glutamate antagonist topiramate, however there has been large heterogeneity in the results (Baldaçara et al., 2016; Johnson et al., 2013; Kampman et al., 2004, 2013; Nuijten et al., 2014; Pirnia et al., 2018; Umbricht et al., 2014). Additionally, based on preclinical data showing a benefit at the neurobiological (reduced glutamatergic transmission, normalization of altered synaptic plasticity, within the PL-NacC pathway) and behavioral level (reduced self-administration reinstatement) (Kupchik et al., 2012; Moussawi et al., 2009), recently, many clinicians have started to prescribe N-acetylcysteine (eg Mucymyst™), as an anti-craving

pharmacotherapy. Initially prescribed for its bronchial fluidizer properties, this promoter of cystine-glutamate exchange still only has mixed efficacy (Stoces et al., 2019).

However, even though these classes of drugs have some potential in reducing drug craving, the results are not uniformly positive. A main impediment on the advancement of pharmacotherapies can simply be attributed to the heterogeneity of the individuals who present to the clinic. Consequently, a focus on personalized medicine (highlighting the individual characteristics of patients coming to the clinic and the consequential efficacy of treatments) needs to be explored in deep before potentially efficacious pharmacotherapies are rejected.

- **Psychosocial therapies**

In contrast to pharmacotherapies, the psychosocial and behavioral treatments for cocaine use disorder aim to not necessarily reduce or eliminate cravings themselves, but reduce the influence of craving by increasing the individuals' self-control and behavioral inhibitory processes. Also in contrast to the pharmacotherapies, many psychosocial therapeutic techniques show relatively good efficacy. The most successful technique to date is a treatment method called contingency management (CM), where the patient is rewarded with a form of reinforcement (money, vouchers, etc) when they achieve a therapeutic goal (Schierenberg et al., 2012). This technique provides a way for the patient to adaptively change their behavior thanks to contingent rewards. However, the mechanism involved in contingency management is not known. As contingency management is a therapeutic method in which the patient learns to inhibit drug seeking behaviors, it is hypothesized that this psychosocial therapy involves continuous recruitment of inhibitory control mechanisms, strengthening the recruitment of the prefrontal cortex in decision-making processes related to drug intake behavior. An additional therapeutic technique that recruits inhibitory control mechanisms is cognitive behavioral therapy (CBT), however it is often used in combination with both pharmacotherapies and other psychosocial therapies such as contingency management (Farronato et al., 2013). Through a combination of pharmacotherapy and psychosocial

therapies, treatment is approached from both “anti-craving” and behavioral inhibition perspectives.

ANIMAL MODELS OF CUD: TOWARD MEASURING MALADAPTIVE COCAINE SELF-ADMINISTRATION

Human addiction is at the core of animal modeling and the clinical view of substance use disorders has changed drastically over the last 30 to 40 years. Over the last 20-25 years, animal model-based preclinical research has attempted to adapt and to take these changes into consideration. The late 1990s and the early 2000s marked a key step forward in the modeling of addiction with the emergence of ‘excessive/escalated’ (Ahmed & Koob, 1998), ‘addiction-like’ (Deroche-Gamonet et al., 2004) or ‘compulsive’ (Vanderschuren & Everitt, 2004) cocaine seeking/taking in rats. Using intravenous self-administration as a model of drug taking, these studies explored the development of excessive and maladaptive cocaine seeking behavior through different behavioral classifications. It merits mention that the work in the early 1990s (Wolffgramm, 1991; Wolffgramm et al., 2000; Wolffgramm & Heyne, 1991) by Wolffgramm and Heyne should be considered a prelude to this paradigmatic shift, as they studied maladaptive drug seeking in a susceptible minority after rats had been given oral drugs for months. Although they were pioneers, this work was widely unrecognized for its novelty.

Animal models of addiction have been challenged due to the innate complexity of addiction in the clinic, but they have tried to quickly adapt and evolve to bring preclinical research closer to what is seen in the clinic. However, the implementation of these new experimental approaches is still limited and preclinical addiction models remain criticized due to their limited success in predicting clinical efficacy of novel therapeutics (Field & Kersbergen, 2020).

Modelling maladaptive self-administration behavior

A common goal of many of these studies was to explore the maladaptive nature of cocaine use after a protracted period of self-administration through different tests (Ahmed, 2010, 2012; Belin-Rauscent et al., 2016; Deroche-Gamonet & Piazza, 2014; Piazza & Deroche-Gamonet, 2013; Vanderschuren & Ahmed, 2013). The most commonly used are the maintenance of drug seeking despite the contingent presence of an aversive stimulus (electric footshock), the presentation of a discrete cue previously associated with an aversive stimulus (electric footshock), or opportunity to self-

administer an alternative natural reinforcer (e.g. sucrose, or more recently, a social partner). In the two first cases, the idea is to test how maladaptive drug seeking is by testing how resistant the seeking patterns are to negative consequences: as it is defined in humans. The basic premise of using an aversive stimulus is that if operant behavior is adaptive, it should decrease if it is punished or associated with a punishment-predicting cue (Jean-Richard-Dit-Bressel et al., 2018). In the last case, the severity of drug seeking is evaluated by testing whether the animal maintains drug seeking when given the opportunity to choose between the drug and a natural reinforcer. It is based on the premise that in a choice procedure, a rodent will choose the most adaptive and naturally preferred reinforcer.

In outbred rats with extensive cocaine self-administration experience, escalation in cocaine use was observed (Ahmed & Koob, 1998) and cocaine seeking was no longer decreased by cues predicting footshocks (Vanderschuren & Everitt, 2004), supporting that maladaptive cocaine seeking could be observed, but also suggesting that cocaine addiction was depending on the level of drug exposure. However, it was also shown that in rats with extensive cocaine self-administration experience, an alternative natural reinforcer (sucrose) still successfully competed with cocaine seeking (Lenoir et al., 2007). In the same vein, a social reinforcer was shown to fully compete with drug self-administration and prevent incubation of drug craving. Venniro et al. (Venniro et al., 2018) demonstrated that operant social reward prevented methamphetamine or heroin drug self-administration independent of sex, drug dose, training conditions, abstinence duration, social housing, or addiction score in Diagnostic & Statistical Manual IV-based and intermittent access models. This choice was lessened by delay or punishment of the social reward but neither measure was correlated with addiction severity. Regarding cocaine, it has been demonstrated that operant social interaction inhibited self-administration and prevented incubation of cocaine seeking in rats trained for self-administration (initially for 2-h/day for 4 days, and then for 12-h/day continuously or intermittently for 8 days), independent of sex (Venniro et al., 2021). This effect has not yet been tested in rats characterized as cocaine addicted-like, however.

For some, these results globally led to questioning if addiction could even be observed in rodents, since a major criterion of addiction in humans (abandonment of natural reinforcers to the benefit of the drug of abuse) was not fulfilled. The fact that concurrent sucrose availability (and now a social reinforcer) was sufficient to divert rats' interest for

cocaine suggested that resistance of seeking to footshock-predicting cues might simply be a response to lack of choice rather than compulsion (Ahmed, 2010). This led others to question extended access and associated escalation in use as a model of addiction (Piazza & Deroche-Gamonet, 2013), suggesting it is rather a model of moderate SUD in which the individuals would remain sensitive to negative consequences and their high motivation being due to adaptations in response to higher levels of intake.

However, two main factors are critical when considering ways to obtain the highest level of translation when considering animal models of addiction:

Individual vulnerability and maladaptive cocaine seeking

All individuals are not at risk for addiction. Taking individual variations into consideration revealed that all rats are not equally at risk for developing a maladaptive cocaine self-administration, including in choice procedures. For quite a long time now, epidemiological data support that only about 15% of vulnerable cocaine users develop behavior that meets the criteria of a severe SUD (Anthony et al., 1994). The DSM in its most recent version (American Psychiatric Association, 2013), acknowledges that not all drug users are vulnerable to SUD. Despite this, the view that development of cocaine addiction is exclusively dependent on drug consumption has persisted until recently, biasing animal modeling.

Many new models of addiction included this bias in their theoretical framework. They consisted of evaluating the consequences of extensive (e.g. extended, i.e. 6 hours/daily session) vs less extensive access (e.g. short 2 hours/daily session) to cocaine self-administration. However, comparison between extended versus short access to self-administration completely overlooks individual susceptibility.

The 3-crit multi-symptomatic addiction model (Deroche-Gamonet et al., 2004) was the first to include the risk dimension of addiction as part of the modeling, applying a threshold/diagnosis for defining an addiction-like behavior and finding evidence for about 20% of 'addicted-like' rats in a population with the same previous protracted cocaine consumption. Group-based models also evidence individual variability: it was revealed that after extended access, some rats [between 9 (Ahmed, 2012) and 14% (Cantin et al., 2010), depending on the reports] do not quit cocaine for sucrose (Ahmed, 2012) or a social reinforcer (Venniro et al., 2021), but also that not all rats

(only about 20%) maintain drug seeking in face of adverse consequences (Pelloux et al., 2007).

Altogether, this leads to a general consensus that protracted exposure to cocaine self-administration is necessary for an addiction-like behavior to be expressed in rats, but that it is not sufficient because it results in this type of behavior only in a minority (Deroche-Gamonet et al., 2004; Deroche-Gamonet & Piazza, 2014; Pelloux et al., 2007; Piazza & Deroche-Gamonet, 2013). This was summarized by BJ Everitt in 2014 when he wrote: 'Experimental animal models of addiction [...] also should reveal individual differences in the propensity to lose control over drug seeking and taking. Not every individual who takes drugs loses that control and compulsively uses drugs despite the adverse or aversive consequences of doing so' (Everitt, 2014).

Influence of cocaine PK and/or PD on maladaptive seeking

The choice ratio between cocaine and an alternative reinforcer was proposed as a safe way to determine how cocaine seeking competes with natural reinforcers and to define maladaptive drug taking behavior (Ahmed, 2012). However, an important factor influences choice behavior: whether a rat chooses cocaine over a natural reinforcer is highly affected by the level of intoxication at the time of choice (Vandaele et al., 2016). The choice between cocaine or sucrose is dependent on the time interval (ITI) between trials, demonstrating that what determines the choice is whether the rat is under cocaine influence or not. This highlights that choice and compulsive-like behavior is sensitive to intoxication, and that pharmacological influence of cocaine serves as an influence on maladaptive seeking patterns that are used to identify addiction vulnerability.

More recently, another aspect of the influence of cocaine pharmacology on choice between the drug and an alternative reward was revealed: the delay to cocaine reward is longer than it is for the nondrug reward (sweetened water). Therefore, rats chose the sweet water reward not because it surpasses cocaine reward in magnitude, but because the sweet reward comes sooner than cocaine reward. Similarly, preference for social reward was decreased by the delay of both rewards, social reward alone, or by increased response requirements for social reward (Venniro et al., 2021). Delay discounting would promote the choice for the earliest reward. Increasing the delay of both rewards or of the natural reward alone reduces the choice for natural reward or even shifts the preferred choice to be the drug.

Individual variations in sensitivity to manipulation of those delays have also been observed, supporting that sensitivity to reward delay or variations in cocaine PK could also play a role in the percentage of animals choosing cocaine over natural reward in these choice procedures (Canchy et al., 2021; Samaha, 2021).

PSYCHOBIOLOGICAL FEATURES OF MALADAPTIVE COCAINE SELF-ADMINISTRATION

Maladaptive behavioral inhibition and discrimination

The 3-crit multi-symptomatic model evaluates cocaine addiction-like behavior using three behavioral tests including two tests involving adaptive behavioral inhibition: one when drug seeking is unbeneficial (seeking when drug is signaled as unavailable) and the other when drug seeking is risky (seeking under foot-shock punishment conditions).

Physiologically, adaptive inhibition, or inhibition of inappropriate actions, relates to the complex and multi-unitary concept of impulsivity (Dalley & Ersche, 2019; Dalley & Robbins, 2017), which is associated with different psychiatric conditions. Notably motor impulsivity (referring to poor motor inhibition to suppress inappropriate, often anticipatory actions (Dalley & Ersche, 2019) is associated with cocaine addiction and is affected by cocaine. Additionally, there is evidence that cocaine use is associated with impaired inhibitory control (Colzato et al., 2007). Both clinical (Ersche et al., 2012) and preclinical data (Belin et al., 2008) support impulsivity as a pre-existing risk factor for cocaine addiction. However, how impulsivity interacts with vulnerability to cocaine addiction both causally, and as a consequence of chronic drug use is unknown (Dalley & Ersche, 2019; Dalley & Robbins, 2017).

Few recent (Herman et al., 2018, 2019) and older (Shlechter & Salkind, 1979) studies in humans have linked impulsivity to discrimination, notably to a reduced ability to discriminate between internal and external signals (Herman et al., 2019; Schlechter & Salkind, 1979) with a possible interplay between internal and external signals (Tuk et al., 2011).

Altered ability to rely on relevant environmental signals to adaptively refrain from drug seeking is a poorly explored hypothesis (Laque et al., 2019; Mihindou et al., 2013; Vanderschuren & Everitt, 2004) both from a longitudinal and in an individual-based

perspective. Preclinical data supports that the maintenance of cocaine seeking in addicted rats involves alteration of control by inhibitory discriminative stimuli (DS).

Addicted-like rats seek for cocaine at a high level during a period in which an external DS signals the drug as unavailable (no drug DS), however they do not differ from non-addicted rats for responding in extinction (no reinforcers, but the presence of a discriminative stimulus signaling drug as available) (Deroche-Gamonet et al., 2004).

Additionally, 70% of these addicted-like rats also show signs of impaired control of seeking by no drug DS after early drug use: after about 20 sessions of self-administration (SA), they develop a reduced ability to inhibit seeking up to the end of signaled no drug periods (Kasanez et al., 2010). 100% of rats showing this specific early pattern of responding in no drug periods become addicted (Deroche-Gamonet & Piazza, 2014), supporting that maladaptive seeking is an early marker of addiction vulnerability. However, it is not clear what causes this initiation of seeking during the signaled no drug period. We make the hypothesis that this early maladaptive seeking during no drug periods relies on internal incentives: notably, vulnerable rats have cocaine seeking behavior that is conditioned to the decrease of brain and/or blood cocaine concentrations which compromises the maintenance of adaptive inhibition.

Maladaptive behavioral inhibition and cocaine PK/distribution

Clinical observations have long established that drug pharmacokinetics influence drug addiction liability: the faster a drug reaches the brain, the higher its addictive potential (Allain et al., 2015). Hence, for a given drug, inter-individual variations in pharmacokinetics (PK) or distribution may influence vulnerability to addiction but are poorly explored.

In controlled conditions, two to threefold variations in peak serum concentrations are observed in response to the same recreational doses of cocaine (Heard et al., 2008; Isenschmid et al., 1992). Cocaine users heterogeneously self-administer cocaine (Roy et al., 2017; Sughondhabirom et al., 2005) and drug metabolism-related genetic variants have been linked with cocaine addiction (Negrão et al., 2013).

In animal models, rats which will later become addicted (3-crit), exhibit a fast pattern of cocaine intake (consuming equivalent amount, but at a faster rate) (Belin et al., 2009) and an increased response to cocaine incentive effects after early cocaine self-administration (Belin et al., 2009). Reproducing such a fast pattern of cocaine intake in a

general population of rats not only increases cocaine incentive effects but also recruits the prelimbic (PL) and fosters a PL/NacC c-Fos clustering more than when comparing to rats with constrained slow pattern (Martín-García et al., 2014).

Consistently, fast cocaine distribution to the brain through short infusion time increases the incentive properties of cocaine and recruits the PL (Allain et al., 2017). Also, compared to continuous self-administration protocols, intermittent cocaine self-administration with repeated short periods of cocaine availability (5 min) followed by longer periods of cocaine unavailability (25 min), are associated with higher motivation for cocaine as measured through progressive ratio, with a burst-like pattern of use, locomotor sensitization, and high cue- or cocaine-induced reinstatement (Allain & Samaha, 2019; Garcia et al., 2020). Like long continuous access (6 hrs/day), intermittent access can result in escalation of drug intake over sessions, however the escalation of intake is not required for incentive sensitization expression. Intermittent access to cocaine, even over short sessions per day and over a short period of time, can promote incentive sensitization independent of escalation, highlighting another key role of cocaine PK in facilitating addiction-like symptoms.

In contrast from what was initially proposed (Ahmed, 2012), the amount of drug is not the critical factor resulting in increased motivation for cocaine (Kawa et al., 2016). Rather, repeated rapid rising and decay in brain cocaine levels, either imposed or self-administered appear crucial to induce high levels of incentive motivation. Whether this cocaine regimen contributes exclusively to increased response to incentive or also to develop maladaptive behavioral inhibition and compulsive-like cocaine self-administration is unknown.

Data support that individual variations in cocaine PK/brain distribution may favor a fast pattern of use, which could promote increased incentive motivation and potential alterations in behavioral inhibition.

NEUROBIOLOGY OF MALADAPTIVE COCAINE SELF-ADMINISTRATION: FOCUS ON THE PRELIMBIC CORTEX AND THE PRELIMBIC TO NUCLEUS ACCUMBENS CORE PATHWAY

Prelimbic cortex

Data has shown the role of the prelimbic cortex as a hub that is used to integrate environmental information for the optimal adaptive response: either promoting or

inhibiting behavior depending on the contingencies (Moorman & Aston-Jones, 2015a). Discrimination processes could be at core of this hub, with external cues (Loureiro et al., 2019), and possibly internal cues/timing (Höhn et al., 2011; Means et al., 2000) being used as discriminative stimuli to initiate or refrain behavior.

Based on reinstatement models (Peters et al., 2009), the PL was long considered to exclusively favor cocaine seeking. Few recent studies manipulating or recording the PL during drug access yielded the opposed conclusion, i.e. an inhibitory control by the PL (B. T. Chen et al., 2013; Limpens et al., 2015; Mihindou et al., 2013; Moorman et al., 2015; Willcocks & McNally, 2013). To date, it has been demonstrated that the PL plays a dual role in controlling cocaine seeking. In the same individuals, PL inhibition reduces cocaine-induced reinstatement but increases cocaine non-adaptive seeking during self-administration (responses during the time-out periods, i.e. 40 sec post-infusion time during which drug is not available) (Martín-García et al., 2014). However, as the prelimbic has afferents and efferents from many other brain regions, identification of the roles of specific pathways could help explain the myriad of behaviors explained by prelimbic activity changes.

Prelimbic/nucleus accumbens core pathway and balance between response to incentive and adaptive inhibition

Data supports that the PL-NacC pathway could be central in controlling the balance between response to incentives and adaptive inhibition over cocaine seeking behavior.

The NacC is involved in mediating response to natural incentives (Mohebi et al., 2019; Sicre et al., 2020), while the PL serves as a hub integrating and dispatching information (Burgos-Robles et al., 2017; Carlén, 2017; Euston et al., 2012; Loureiro et al., 2019; Otis et al., 2017; Sangha et al., 2014) for adaptive expression of motivated behaviors (Balleine & O'Doherty, 2010; Y.-W. Chen et al., 2016; Euston et al., 2012; Moorman & Aston-Jones, 2015b; Otis et al., 2017). While still scarce and indirect, data suggest that the PL could adaptively modulate NacC-mediated incentive effects, as a function of internal state (Aitken et al., 2016; Moscarello et al., 2010) and external information (Loureiro et al., 2019). The PL's modulation of NacC-mediated incentive effects through spatial cues (Cui et al., 2018; Malagon-Vina et al., 2018; Rich & Shapiro, 2009) is more clear. Food-rewarded spatial tasks involve the NacC, and switching between self and spatial navigation strategies (Malagon-Vina et al., 2018; Rich & Shapiro, 2009) to reach the

reward depends on PL control of the NacC in a dopamine-dependent manner (Cui et al., 2018).

Regarding cocaine, research has mainly focused on increased response to drug incentives, and the PL projection to the NacC has been revealed to be an important pathway (McGlinchey et al., 2016). In the context of controlled cocaine self-administration, as proposed above for natural reinforcers, the PL could serve as a hub involved in controlling NacC-mediated incentive effects by balancing the cost-benefit analysis in engaging in cocaine seeking. The PL-NacC pathway would therefore be central in adaptive cocaine seeking by balancing the response to drug incentives with adaptive behavioral inhibition and at the core of maladaptive seeking in addiction-vulnerable users.

Susceptibility of the PL-NacC pathway to cocaine PK/distribution and relation to addiction vulnerability

The connectivity between the PL and NacC seems to be influenced by pharmacokinetic influences. Rats allowed to self-administer cocaine at high frequency showed increased cocaine-induced incentive effects and enhanced c-Fos expression in the PL and infralimbic (IL) areas of the mPFC, the basolateral amygdala, and the NacC and shell. Correlational analysis of c-Fos revealed that the PL was a critical node strongly correlated with both the IL and NacC in these fast cocaine-taking rats (Martín-García et al., 2014).

Additionally, in an intermittent access protocol, rapid injections (5s vs 90s) promoted psychomotor sensitization, potentiated incentive motivation for cocaine, and was accompanied by enhanced functional activity of metabotropic glutamate group II receptors (mGluR2/3s) in both the PL and NacC. Activation of mGluR2/3s with LY379268 preferentially decreased the motivation to take cocaine in rats previously exposed to rapid drug injections (Allain et al., 2017).

Regarding addiction vulnerability as measured through the 3-crit model, LY379268 was also shown to reduce reinstatement in rats exhibiting addiction-like behavior highlighting that the same mechanisms that result from rapid injections may also be induced by a self-imposed fast pattern of intake that is present in rats exhibiting addiction-like behavior (Cannella et al., 2013).

Switching to synaptic plasticity (mGluR2/3 and NMDA-dependent LTD) within the two structures, it was shown that addiction-vulnerable and addiction-resistant rats differed

in adaptations within the pathway over the course of the transition to addiction-like behavior.

After early cocaine use (17 self-administration sessions), NMDA-LTD in the NacC was abolished in both addiction-vulnerable and addiction-resistant rats, while mGluR2/3-LTD in the PL was unaffected. When addiction-like behavior was expressed, NMDA-dependent LTD in the NacC and mGluR2/3-LTD in the PL were only altered in the 3-crit rats. Additionally, 3-crit rats expressed less mGluR2/3 in the PL as compared to 0-crit rats. It was proposed that protracted loss of plasticity in the NacC could be responsible for the loss of mGluR2/3-LTD specifically observed in the PL in 3-crit rats, even though mGluR2/3-LTD in the PL remains unaltered after early training in both vulnerable and resistant rats. Differently, expression of mGluR2/3-dependent LTD in the NacC was altered in both 0-crit and 3-crit rats suggesting that it is more likely related to protracted cocaine use but not necessarily directly related to expression of addiction-like behavior.

These studies show that the PL and NacC activity is altered as a function of intake pattern and vulnerability to addiction. This pathway serves as a promising target for uncovering synaptic plasticity changes promoting the disruption between incentive motivation and adaptive behavioral inhibition.

INFLUENCE OF SEX- HORMONAL INFLUENCES

Vulnerability to addiction

The influence of sex on vulnerability to cocaine addiction is still quite unclear. Clinical studies exploring the contribution of sex in the domain of cocaine use disorder and craving have not reached clear conclusions, leading to continued uncertainty of the presence of sex differences in CUD (Nicolas et al., 2022).

In preclinical models, many studies have shown more frequent sex differences, with female rats acquiring cocaine self-administration quicker with concurrent increases in the reinforcing, psychomotor-sensitizing, and incentive motivational effects of cocaine (Algallal et al., 2020). Notably, increased incentive-motivational effects of cocaine are observed during the estrus phase of the menstrual cycle, enforcing an important link between differences in incentive-motivation during the different phases. Regarding maladaptive seeking (that incorporates a level of behavioral inhibition that is less present in tests researching incentive motivation), in an intermittent access model of self-administration females had significantly more maladaptive seeking patterns (seeking

during the drug unavailable period) than males (Kawa & Robinson, 2019). There is a substantial gap in the literature regarding both the dynamics of maladaptive seeking and inhibitory control in females, and secondly, how these differences may be sensitive to different phases of the menstrual cycle. While a lot of research is focused on sex differences as a consequence of the effect of the menstrual cycle on dopaminergic signaling, there is also a role of the estrous cycle on the glutamatergic system that could help explain some of the addiction-like behavioral deficits that are observed to a more detrimental extent in females than males (Calipari et al., 2017; Giacometti & Barker, 2020).

Cocaine pharmacokinetics and pharmacodynamics

As mentioned previously, in humans, variations in peak cocaine concentration exist between individuals, however this effect is also strongly sensitive to sex differences, with higher maximum cocaine concentrations in females (Coe et al., 2018). Additionally, different phases of the menstrual cycle are associated with differences in metabolism of cocaine. Butyrylcholinesterase activity levels, a plasma esterase that is involved in the catabolism of cocaine, has been shown to be lower in the luteal phase than the follicular phase (Vaisi-Raygani et al., 2013). Lastly, peak cocaine concentration is achieved quicker in the follicular phase than the luteal phase suggesting that menstrual cycle phases strongly contribute to differences in females, which could explain why women in the follicular phase report better subjective experiences with cocaine administration (Evans et al., 2002; Lukas et al., 1996). Further investigation of pharmacokinetic differences between sexes is relatively underexplored in human studies.

Preclinical studies examining sex differences of cocaine pharmacokinetics are also exceptionally rare, but there are some studies that indicate estrogen and progesterone can affect cocaine distribution in the brain (Niyomchai et al., 2006), likely through changes of blood brain barrier permeability through modulation of the endothelial cells and/or tanocytes (Prevot et al., 2018; Wilson et al., 2008).

Neurobiology

In clinical research, sex differences in neurobiological changes as a function of cocaine use disorder have been identified. Specifically, females may be more resistant to the neural damage that is caused by continued cocaine use (Andersen et al., 2012). However, as neurobiological sex differences innately exist and are sensitive to hormone changes, more progress needs to be done to understand how differences in CUD and the related behavioral handicaps relate to neurobiological changes.

Preclinical research has highlighted that there is a sex-dependent role of the PL that differentially predicts impulsive action in male rats, but not female rats. Even though this

relationship between PL activity and impulsivity exists before cocaine self-administration, the relationship becomes much more significant after cocaine exposure (Moschak & Carelli, 2021a).

Regardless of an unclear picture of sex differences in cocaine addiction, there is evidence supporting sex differences within cocaine pharmacokinetics and the neurobiological changes associated with cocaine use disorder. The mechanisms that occur in the transition from controlled to maladaptive drug intake is likely different, emphasizing the importance of exploring the sex differences in maladaptive seeking behavior, pharmacokinetic differences, and neurobiological dysfunction in a longitudinal way.

GENERAL HYPOTHESIS AND OBJECTIVES

Defaults in adaptive behavioral inhibition may relate to an altered ability to rely on relevant environmental discriminative signals due to a progressive disengagement of the PL over NacC-mediated incentives, which would be favored by early spontaneous fast pattern of use promoted by specific influence of cocaine pharmacokinetics (PK) or distribution.

My thesis specifically focuses on understanding the psychobiological mechanisms of the emergence of maladaptive behavioral inhibition over cocaine seeking. Within my research, I use the self-administration protocol used in the rat CUD model pioneered by our lab. In this model, 15 to 20% of rats develop addiction-like behavior after protracted intravenous self-administration (SA). Critically, after early cocaine SA, a default in adaptive inhibition over cocaine seeking is predictive of the transition to addiction.

Through three principal objectives, my thesis work aims at evidencing psychobiological bases of this early predictive marker of addiction vulnerability.

1. **Dissect the psychopharmacological components that influence expression of maladaptive seeking behavior** in rats that are identified as vulnerable or resistant to developing cocaine addiction from a well-established pre-clinical model of cocaine use disorder.

I hypothesized that maladaptive seeking results from an altered control of behavior by internal versus external discriminative signals. During the ND period, cocaine levels decrease. In vulnerable rats, the decrease in cocaine concentration serves as an incentive to drive re-initiation of seeking despite the presence of the inhibitory DS.

I tested the hypothesis that the maladaptive seeking pattern will not emerge if cocaine concentration is maintained throughout the duration of the period through non-contingent cocaine injections. Additionally, I tested how a prolongation of the no drug period affects seeking behavior in both resistant and vulnerable rats. To evaluate whether the lost adaptive inhibitory control of the no drug DS on seeking observed in vulnerable rats is drug state dependent, I also evaluated it in a drug free state. Lastly, to study the balance between response to incentive and adaptive inhibition, I tested the influence of adaptive inhibition on cocaine-induced incentive effects as measured through cocaine-induced reinstatement in vulnerable and resistant rats.

2. **Determine if there are differences in cocaine pharmacokinetics and distribution** in vulnerable and resistant populations and how they relate to maladaptive seeking behavior expression.

I hypothesized that maladaptive seeking patterns may emerge due to differences in cocaine pharmacokinetics in vulnerable and resistant populations. To explore this, intracerebral quantification of cocaine and its metabolites were performed after equal administration of cocaine and related to previous self-administration behavior. Additionally, cocaine metabolism was analyzed as a function of self-administration behavior through exploration of plasma butyrylcholinesterase activity.

3. **Identify differences in prelimbic cortex (PL) functioning in rats** expressing maladaptive seeking compared to those who adaptively control cocaine-seeking behavior.

Focusing on the PL, I implemented fiber photometry in combination with intravenous cocaine self-administration to evaluate the activity of PL principal neurons through calcium imaging. Notably we created an open-source data analysis pipeline for fiber photometry coupled with operant behavior for optimal exploration of calcium transients in response to self-administration-associated events. PL activity was analyzed as a function of maladaptive seeking patterns as well as levels of intoxication to uncover differences in PL functioning in behaving animals.

Through these complementary objectives, I established a clearer relationship between addiction vulnerability as a function of psychopharmacological influences, highlighting key differences in

PL functioning between the two populations that are dependent on internal state and may strongly contribute to addiction vulnerability.

COMMON MATERIALS AND METHODS

SURGERY

A silastic catheter (internal diameter = 0.28 mm; external diameter = 0.61 mm; dead volume = 12 μ l) was implanted in the right jugular vein under isoflurane anesthesia (induction: 5%, maintenance: 3%). The proximal end of the catheter was inserted into the right atrium, passed under the skin, and the base emerged from the mid scapular region. Rats were treated post-op with Metacam (1 mg/kg, SC) and allowed to recover for 5 to 9 days after surgery before self-administration began.

DRUGS

Cocaine hydrochloride (Cooper, France) was dissolved in 0.9% physiological saline to result in a cocaine hydrochloride solution.

INTRAVENOUS SELF-ADMINISTRATION APPARATUS

Self-administration occurred in a room that contained 40 self-administration apparatuses. The self-administration setups were composed of plexiglas and metal (Imetronic, Marcheprime, France). Each chamber (40 cm long x 30 cm width x 52 cm high) was encased within a larger opaque box equipped with exhaust fans that assured air circulation and masked background noise (**Figure 1**). During the sessions, animals were placed in a chamber where their chronically implanted intra-jugular infusion catheter was connected to a pump-driven syringe. Two holes, located on opposite sides of the chamber at 5 cm from the grid floor, were used to record responding. The chambers were equipped with a blue cue light that was used to signal drug availability (drug DS) (1.8 cm in diameter) located on the left side of the opposite wall from the active hole at 33 cm from the grid, a white house light at the top of the chamber that was used to signal drug unavailability, (no drug DS) and a white cue light (1.8 cm in diameter) located 9.5 cm above the active hole that signaled a cocaine injection (cocaine CS). Experimental contingencies were controlled and data was collected with PC-windows-compatible software (Imetronic, Marcheprime, France).

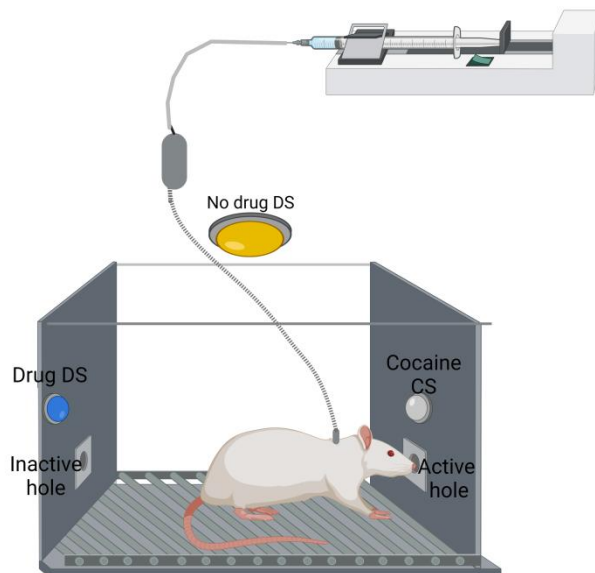


FIGURE 1: Self-administration apparatus- Operant chambers were equipped with two holes at opposing sides of the chamber. The conditioned stimulus (Cocaine CS) was located above the active hole while the drug discriminative stimulus (Drug DS) was located on the wall where the inactive hole was located. The no drug discriminative stimulus (No drug DS) was located on the ceiling of the chamber. Cocaine injections were done by activation of a pump loaded with a syringe containing a cocaine solution suitable for IV delivery. The pump was connected to the rat by silastic tubing (encased in metal) running from the syringe to the catheter port on the scapular region of the rat. Created with BioRender.com.

INTRAVENOUS SELF-ADMINISTRATION PROCEDURE

Basal self-administration session

All experiments were performed in the dark phase of the light/dark cycle. The daily sessions consisted of three drug periods (40 min) separated by two no drug periods (15 min) (**Figure 2A**). Drug periods were characterized by illumination of the blue cue light (drug DS) while no drug periods were characterized by illumination of the chamber by the white house light (no drug DS). Inactive nose-pokes during the duration of the sessions were recorded but had no scheduled consequences and active nose-pokes during the no drug period were recorded but had no scheduled consequences. Animals were trained on an FR3 ratio of responding for the first three days of self-administration (max infusions 25, 25, 30, respectively), then continued on

an FR5 ratio of responding for the duration of the experiment (max infusions 30 for the first day at FR5, then 35 for the remainder of the experiment). Once the animals reached the necessary number of nose-pokes for infusion, the white cue light (cocaine-associated cue- cocaine CS) illuminated for four seconds with the pump being activated (delivering cocaine at a dose of 0.8 mg/kg/infusion: infusion volume=46 μ L over two seconds) starting one second after the illumination of the white cue light. Then, both the white cue light (cocaine CS) and blue light (drug DS) were turned off during the time out period (40 seconds). Active and inactive responding during the time out period was recorded but had no scheduled consequences.

DATA AND STATISTICAL ANALYSIS

Acquisition of intravenous cocaine self-administration behavior

Variables of interest were: Total active and inactive nose-pokes during the drug and total active and inactive nose-pokes during the no drug periods for each session, as well as total injections received per session and average inter-infusion interval over the session.

Sex differences were explored by using repeated measures ANOVAs on the total injections and total inter-infusion interval during acquisition with the session (self-administration session) as a within-subject factor and the sex (male vs female) as a between subject factor.

Self-administration behavior was analyzed by using repeated measures ANOVAs on the total active nose-pokes during the drug period, and/or active nose-pokes during the no drug period, and/or inactive nose-pokes during the drug period, and/or inactive nose-pokes during the no drug period, with sessions (self-administration session), holes (active vs inactive), and periods (drug vs no drug) as within-subject factors and maladaptive seeking groups (high vs low) as a between-subject factor when relevant. Significant main effects or interactions were explored by pairwise comparisons of means using the Fisher LSD post-hoc test.

Stability in cocaine intake was confirmed by using repeated measures ANOVA on the total number of injections per self-administration session with the session (self-administration session) as a within-subject factor. Significant main effects of the session were explored by pairwise comparisons of means using the Fisher LSD post-hoc test and stability in intake was determined by non-significant differences between session days.

Criteria for identification of maladaptive seeking rats (maladaptive group)

Variables of interest were: Mean total nose-pokes in the active hole during the first no drug period over three consecutive sessions

Maladaptive seeking groups (high and low) were determined by performing a median split of the average active hole responses during the first no drug period. Rats with seeking patterns equal or above the median were characterized as high seekers, while rats that had seeking patterns lower than the median were characterized as low seekers.

Group comparisons

Comparison of basal behavior between maladaptive seeking groups (high vs low) at the time of identification was performed by unpaired t tests, comparing the no drug responding rates, injections, inter-infusion interval (III), brain cocaine concentration, and/or plasma BChE levels between the two subpopulations.

Correlational analysis

Pearson's correlation analyses were used to explore correlations between continuous variables of interest.

All statistical analysis was performed using the STATISTICA 14.0.0.15 (2020) system (TIBCO Software, CA, USA).

All graphs were done using GraphPad Prism. The results are presented as mean \pm SEM.

Differences were considered significant at $p < 0.05$.

CHAPTER 1: PSYCHOPHARMACOLOGICAL CONTRIBUTIONS TO MALADAPTIVE COCAINE SEEKING

As discussed previously, maladaptive cocaine seeking (seeking cocaine while disadvantageous or risky) is a main feature of cocaine addiction. Maladaptive seeking is proposed to result from increased response to incentives (drug cues, drug itself, stress) and reduced adaptive inhibitory control. However, the factors influencing adaptive inhibitory control are rarely disentangled.

After early cocaine self-administration, rats which seek for cocaine during periods of signaled drug unavailability (ND periods) are at risk for transition to addiction-like behavior. This maladaptive seeking in absence of experimenter-delivered incentives allows us to question factors influencing adaptive behavioral inhibitory control over disadvantageous cocaine seeking.

As previously mentioned, we hypothesize potential disruptions in discriminative ability to be involved in altered adaptive inhibitory control. We aimed to understand and explore the ability of an external DS signaling drug seeking as useless to control cocaine seeking in addiction vulnerable and resistant rats as a function of drug state and external incentives.

During the ND period, cocaine levels decrease. We make the hypothesis that vulnerable rats are not able to maintain inhibition over seeking because their behavior is driven by the decrease in cocaine concentration, which serves as an internal incentive (or discriminative signal) announcing that it is time to reinitiate seeking.

Based on this hypothesis, the maladaptive seeking pattern should not emerge if cocaine concentration is maintained throughout the duration of the period through non-contingent cocaine injections. In parallel, we explored how prolonging the duration of the no drug period affects seeking behavior in both resistant and vulnerable rats.

To evaluate whether the ND DS had acquired an adaptive inhibitory value in the vulnerable rats (that would be more sensitive to incentive or discriminative effects of a decrease in cocaine concentration), we tested its effect on seeking behavior while rats are in a drug free state, without incentives (other than the self-administration context).

Lastly, and based on the observation that the ND DS has indeed acquired an adaptive inhibitory value also in the vulnerable rats, we explored this adaptive inhibitory value when seeking behavior is experimentally incited by non-contingent cocaine (reinstatement). We compared cocaine-induced reinstatement under Drug (D) and ND DS, which also permits testing the

balance between response to incentive and adaptive inhibition, a major clinical issue in users with problematic cocaine use.

For the sake of sex comparison, a cohort of female rats was tested for most manipulations.

MATERIALS AND METHODS

SUBJECTS

Sprague Dawley rats (manipulation 1: ♂: n=62, ♀: n=19; manipulation 2: ♂: n=25; manipulation 3: ♂: n=24, ♀: n=19; manipulation 4: ♂: n=86, ♀: n=14) weighing 250-300g at their arrival were single housed in a temperature ($22 \pm 1^\circ\text{C}$) and humidity ($60 \pm 5\%$) controlled vivarium on a reverse-light cycle (ON 19h00, OFF 7h00). Food and water were provided *ad libitum*. Rats were habituated to the vivarium for 1 and a half weeks before surgery and were handled daily before experimentation began.

For surgery, drugs, and intravenous self-administration apparatus, see common methods.

SPECIFIC INTRAVENOUS SELF-ADMINISTRATION PROCEDURE

Basal self-administration sessions were as described in the common methods.

Manipulation 1: Non-contingent infusion (NCI) session

The non-contingent infusion session was identical to the basal self-administration session other than two non-contingent infusions that occurred during the first no drug period (**Figure 2B**). The two infusions occurred five and ten minutes into the first no drug period, respectively. Each dose delivered was identical in terms of volume and concentration to the dose that the animal self-administers during the drug periods (0.8 mg/kg in 46 μL /2sec) but the injection was not signaled by the conditioned stimulus as it is during the drug period.

Manipulation 2: Long ND period SA session

The SA session with a long ND period consisted in a normal drug period (40 min) followed by a 90 minute no drug period (**Figure 2C**), rather than a 15 minute no drug

period. Like during the other no drug periods, active and inactive nose-pokes were recorded but had no scheduled consequences.

Manipulation 3: No drug pre (ND pre) session

The no drug pre session was identical to the basal self-administration sessions but with an additional no drug period before the first drug period (**Figure 2D**). Like during the other no drug periods, active and inactive nose-pokes were recorded but had no scheduled consequences.

Manipulation 4: Cocaine-induced reinstatement session

A cocaine-induced reinstatement session lasted 181 minutes. The first 60 minutes were an extinction period where the drug discriminative stimulus was illuminated. At the end of the extinction period, either the no drug discriminative stimulus started to be highlighted (no drug DS group) or the drug discriminative stimulus continued being illuminated (drug DS group). One minute later and keeping these light conditions, four increasing volumes (23, 46, 92 and 184 μ L i.v.) of the cocaine solution (corresponding to 0.2, 0.4, 0.8, 1.6 mg/kg of cocaine) were delivered non-contingently over two hours, one every thirty minutes (**Figure 2E and 2F**). Active and inactive nose-pokes throughout the reinstatement session were recorded but had no consequence, nor did they result in contingent drug delivery.

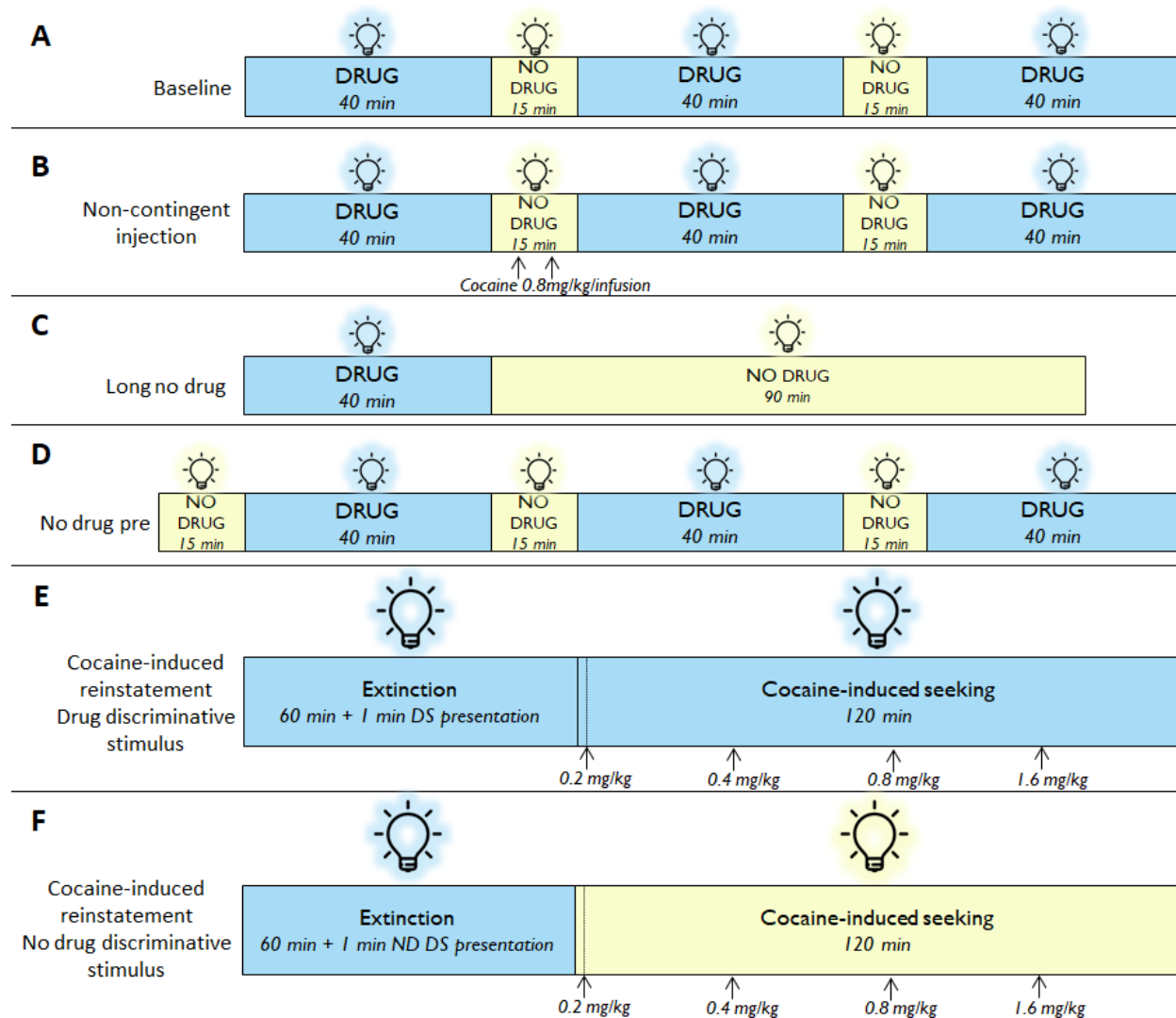


FIGURE 2: Self-administration sessions- A. Baseline (or basal) self-administration sessions consisted of three forty-minute drug periods separated by two fifteen minute no drug periods. B. A non-contingent injection session was a basal self-administration session apart from the first no drug period where two unsigned non-contingent injections occurred at the fifth and tenth minute of the first no drug period. C. The long no drug session consisted of a normal 40 min drug period followed by a 90 minute no drug period rather than a 15 minute no drug period. D. The no drug pre session initiated with a 15 minute no drug period then continued to a basal session. E and F. Cocaine-induced reinstatement sessions consisted of 60-minute extinction periods and then 1 minute of the DS presentation. In the drug DS condition, there was no change (E) while in the no drug DS condition, the light was switched to the ND DS for one minute. (F). Afterwards, non-contingent injections of cocaine at increasing dose occurred every 30 minutes (0.2 mg/kg, 0.4 mg/kg, 0.8 mg/kg, and 1.6 mg/kg) and the DS conditions were kept as described for the last min of the extinction phase.

EXPERIMENTAL TIMELINE

An overview of the experimental timeline is presented (**Figure 3**). If manipulations consisted of multiple cohorts, analysis was performed on the self-administration behavior to ensure that the acquisition of self-administration (total number of injections over the whole SA session, rate of intake during the whole SA session, and total no drug active nose-pokes during the whole SA session) over the first 16 days were not subjected to an experiment effect.

Adaptive inhibition as a function of drug state

Manipulation 1 (Non-Contingent Injection during ND period - NCI)

Males

Manipulation 1 was performed on two cohorts (n=62). In the first cohort (n=36), the NCI session occurred on session 32. In the second cohort (n=26), the NCI session occurred within sessions 17-26.

Between the two cohorts, no experimental effect was observed for the total number of injections [$F(1,60)=1.0124$, $p=0.31836$], rate of intake [$F(1,60)=0.91096$, $p=0.34369$], and total no drug active nose-pokes [$F(1,60)=0.41140$, $p=0.52370$] per session for sessions 1-16.

Females

Manipulation 1 was performed on a single cohort (n=19). The results for females were separated by sex as there were significant differences in acquisition of injections between males and females [n=81, ANOVA, sex effect: $F(1,79)=6.8433$, $p=0.0107$].

The NCI session occurred on session 28 for all rats.

Manipulation 2 (SA session with Long ND period)

Males

Manipulation 2 was performed on a single cohort (n=25) between sessions 21-28.

Females were not tested for this condition.

Manipulation 3 (ND period in a drug free state – ND pre session)

Males

Manipulation 3 was performed on a single cohort (n=24) after they underwent an NCI session. All ND Pre sessions occurred within sessions 18 to 34 (based on level of ND responding stability) and each rat underwent 4 ND pre sessions. The ND pre sessions used for analysis were the 2nd, 3rd, and 4th ND Pre session.

Females

Manipulation 3 was performed on a single cohort (n=19). All ND Pre sessions occurred within sessions 31 to 34 (based on level of ND responding stability) and each rat underwent 4 ND pre sessions. The ND pre sessions used for analysis were the 2nd, 3rd, and 4th ND Pre session.

Adaptive inhibition over incited cocaine seeking

Manipulation 4 (Effect of ND DS on cocaine-induced reinstatement)

Males

Manipulation 4 was performed on three cohorts (n=86). Between the three cohorts, no experimental effect was observed for the total number of injections [F(2,83)=0.33672, p=0.71508], rate of intake [F(2,83)=0.95802, p=0.38785], and total no drug active nose-pokes [F(2,83)=1.5411, p=0.22021] per session for sessions 1-16.

In all three cohorts (n=31, n=28, and n=27), reinstatement occurred on session 23. Reinstatement was designed between-subject, where one animal had exposure to cocaine-induced reinstatement under a single DS. Before reinstatement, groups to be tested under Drug or No Drug DS were confirmed to be equivalent in total no drug responding and cocaine intake patterns (injections and III).

Females

Manipulation 4 was performed on a single cohort (n=14). The results for reinstatement were separated by sex as there were significant differences in acquisition between males and females. Reinstatement occurred on session 23 and was between-subject (as it was for the males) with establishment of

equivalent total no drug responding and intake patterns (injections and III) within groups before the reinstatement session.

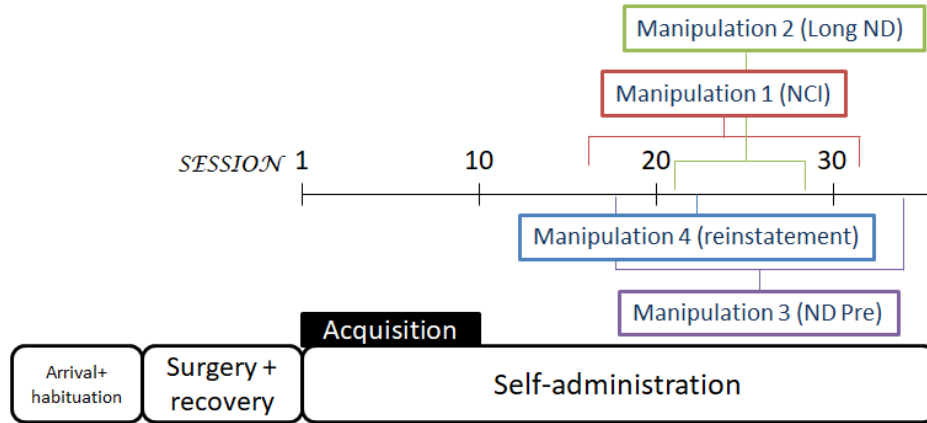


FIGURE 3: Experimental timeline- Chapter 1- In all experiments, rats had at least 16 sessions for self-administration acquisition. Manipulation 1 (NCI) occurred from sessions 17-32. Manipulation 2 (Long ND) occurred between sessions 21-28. Manipulation 3 (ND period Pre session- ND Pre) occurred from sessions 18-36. Manipulation 4 (reinstatement) occurred on day 23.

SPECIFIC DATA AND STATISTICAL ANALYSIS

Identification of maladaptive seeking rats

Rats were identified as described in the common methods as High and Low seeking rats. The sessions considered for the classification were different according to the considered manipulation (see specificities of each manipulation in results).

Group comparisons

Rats identified as High and Low seeking rats were compared as described in the common methods.

Intravenous cocaine self-administration behavior

Acquisition of cocaine intravenous self-administration was analyzed as described in the common methods.

Non-contingent injection (manipulation 1)

Variables of interest were: Mean active nose-pokes during the first 5 minutes and last ten minutes of the first no drug period of the three sessions occurring the three days before the non-contingent injection session, as well as active nose-pokes during the first 5 minutes and last ten minutes of the non-contingent injection session.

Active nose-pokes during the first five and last ten minutes of the non-contingent injection session were compared to the mean active nose-poking activity during the first five minutes and last ten minutes of three basal no drug periods using a repeated measures ANOVA with condition (baseline or NCI session) and time (first 5 minutes vs last 10 minutes of NCI ND period) as within-subject factors and maladaptive behavior group (high vs low) as between-subject factors when relevant. Significant main effects or interactions were explored by pairwise comparisons of means using the Fisher LSD post-hoc test.

Long ND session (manipulation 2)

Variables of interest were: Active nose-pokes during 15 minute bins during the long ND session

Active nose-pokes during the long ND session was analyzed using repeated measures ANOVA with time (15 minute bins) as a within-subject factor and maladaptive behavior group (high vs low) as a between-subject factor. Significant main effects or interactions were explored by pairwise comparisons of means using the Fisher LSD post-hoc test.

ND Pre self-administration session (manipulation 3)

Variables of interest were: Mean total active nose-pokes during the drug free ND period (ND pre) and the intoxicated ND period (ND1) over three consecutive ND Pre sessions.

The two variables of interest were compared using repeated measures ANOVAs with condition (ND pre or ND1) as a within-subject factor and maladaptive behavior group (high vs low) as between-subject factors when relevant. Significant main effects or interactions were explored by pairwise comparisons of means using the Fisher LSD post-hoc test.

Cocaine-induced reinstatement (manipulation 4)

Variables of interest were: Active nose-pokes and inactive nose-pokes during extinction, and active and inactive nose-pokes for the 30 minutes following each dose administered during the reinstatement session.

Active and inactive nose-poking during extinction in the reinstatement session were analyzed using a repeated measures ANOVA with hole (active or inactive) and time (5 minute bins during extinction) as within-subject factors and discriminative stimulus group (drug DS vs no drug DS) and maladaptive seeking group (high vs low) as between-subject factors when relevant. Significant main effects or interactions were explored by pairwise comparisons of means using the Fisher LSD post-hoc test.

Active and inactive nose-poking during cocaine-induced reinstatement was analyzed using a repeated measures ANOVA with dose (dose administered) and hole (active or inactive) as within-subject factors and discriminative stimulus group (drug vs no drug) and maladaptive behavior group (high vs low) as between-subject factors when relevant. Significant main effects or interactions were explored by pairwise comparisons of means using the Fisher LSD post-hoc test.

RESULTS

ADAPTIVE INHIBITION AS A FUNCTION OF DRUG STATE

Manipulation 1: Effect of non-contingent cocaine injections on maladaptive seeking

Acquisition of self-administration behavior

Males

Rats acquired cocaine self-administration as shown by both the significant discrimination between the active and inactive holes [ANOVA, hole effect: $F(1, 60)=170.89$, $p<0.0001$] increasing over sessions [hole x session effect: $F(15, 900)=15.202$, $p<0.0001$] (**Figure 4A**) and an increase in the number of cocaine injections per session which achieved stability in the population by day 10 [N=62, ANOVA, session effect: $F(15, 900)=45.644$, $p<0.0001$] (**Figure 4B**).

Regarding no drug periods, we show here the first one (as this is the one that will be used for rats' classification as high and low maladaptive seekers). In this period, a higher number of responding in the active hole was also observed [ANOVA, hole effect: $F(1, 56)=27.500$, $p<0.0001$] with fluctuation over the sessions [hole x session effect: $F(15, 840)=2.686$, $p<0.0001$] (**Figure 4C**).

Females

Rats acquired cocaine self-administration as shown by both the significant discrimination between the active and inactive holes [ANOVA, hole effect: $F(1, 17)=58.358$, $p<0.0001$] increasing over sessions [hole x session effect: $F(16, 272)=3.1712$, $p<0.0001$] (**Figure 4D**) and an increase in the number of cocaine injections per session which achieved stability in the population by day 7 [N=19, ANOVA, session effect: $F(16, 272)=28.562$, $p<0.0001$] [Fisher LSD post-hoc] (**Figure 4E**).

Regarding no drug periods, we show here the first one (as this is the one that will be used for rats' classification as high and low maladaptive seekers). In this period, a higher number of responding in the active hole was also observed [ANOVA, hole effect: $F(1, 14)=12.619$, $p=0.0032$] however, unlike males, fluctuation did not occur over the sessions [hole x session effect: $F(16, 224)=1.3954$, $p=0.1452$] (**Figure 4F**).

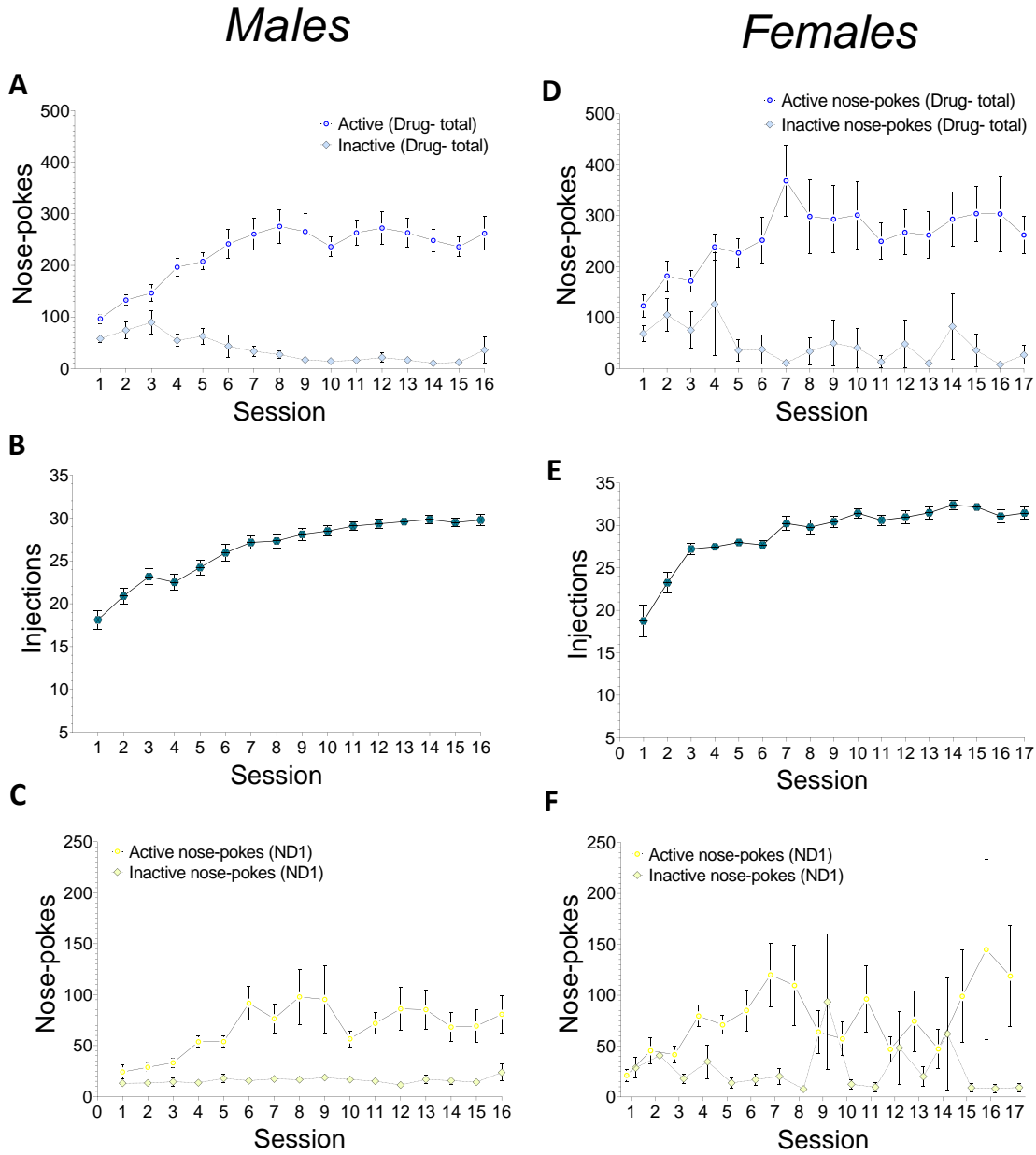


FIGURE 4: Acquisition: Manipulation 1- Males: A. There was discrimination between the active and inactive holes in the drug period over the acquisition period. B. Stability in cocaine injections taken per session was achieved by day 10. C. There was discrimination between active and inactive holes in the first drug period over the acquisition period.

Females: D. There was discrimination between the active and inactive holes in the drug period over the acquisition period. E. Stability in cocaine injections taken per session was achieved by day 10. F. There was discrimination between active and inactive holes in the first drug period over the acquisition period.

Identification and classification of maladaptive seeking groups

Males

Manipulation 1 (non-contingent injection) was done on multiple cohorts that had slightly different protocols, therefore classification of high and low seekers was done on a median split of the average ND responding from days 15-17 in the first cohort and days 14-16 in the second cohort (before differences in protocol emerged). The difference in days used to establish the groups was due to the non-contingent injection occurring at session 17 in the second cohort for a selection of animals.

As previously mentioned, in acquisition, responding and discrimination evolved over time in the drug and no drug periods. In the first no drug period, this relationship differed between high and low seekers [No drug 1: hole x session x maladaptive group effect: $F(15, 840)=2.8936$, $p<0.001$] indicative of the progressive development of maladaptive seeking in active hole by high seeker rats over the 'acquisition' phase of self-administration (**Figure 5**).

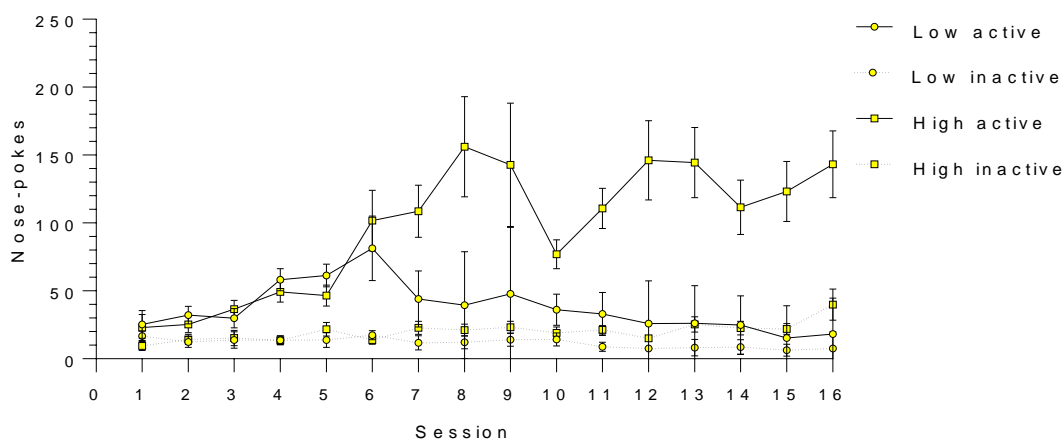


FIGURE 5: Acquisition: Manipulation 1- Evolution of no drug 1 responding in high and low seekers- Males: Discrimination evolved differently in active and inactive holes in high and low seekers in the first no drug period. Notably, we observed that maladaptive seeking in high rats started to occur at session 7 while the low rats diminished active seeking over time and eventually no longer discriminated between active and inactive holes.

This difference was also observed for the drug periods [Drug: hole x session x maladaptive group effect: $F(15, 900)=2.2357$, $p<0.005$], while not impacting the injection

patterns over the acquisition period [maladaptive group x session effect: $F(15, 900)=.73878$, $p=.74594$], and only tending to impact the total number of injections earned [maladaptive group effect: $F(1, 60)=2.8614$, $p=.09591$].

As expected, total no drug nose-pokes were significantly different in the high ($n=35$) and low ($n=27$) groups ($p<0.001$) during the sessions used to characterize them as high and low seekers (**Figure 6A**), but average injections during the entire sessions (**Figure 6B**) or the average inter-infusion interval of the sessions (**Figure 6C**) did not differ (injections $p=0.082785$, III $p=0.071891$).

Females

Classification of maladaptive seeking groups was performed on a median split of the average ND responding from days 15-17.

In the drug period, the evolution of responding (hole x session effect) previously observed was not different between high and low seekers [Drug: hole x session x maladaptive group effect: $F(16, 272)=1.2869$, $p=0.2047$].

In the no drug period, responding only tended to evolve differently between high and low seekers over acquisition [No drug 1: hole x session x maladaptive group effect: $F(16,224)=1.517$, $p=0.095$]. Although not significant due to a limited number of rats, the profile was similar as the one observed for males, with high seeker rats showing higher level of active nose-pokes than low seeker rats starting at session 7 (data not shown).

The number of injections was higher in the high seekers than the low [ANOVA, maladaptive group effect: $F(1, 17)=18.039$, $p<0.001$] however both groups had a similar trajectory of acquisition [maladaptive group x session effect: $F(16,272)=1.5617$, $p=0.07889$].

Between the high ($n=11$) and low ($n=8$) groups, as expected the average total active no drug nose-pokes was significantly different on the sessions used to identify them ($p<0.05$) (**Figure 6D**). Different from the male rats, and consistent with the acquisition data, on these sessions, high or low female seekers differed for the average number of total injections (**Figure 6E**) and the average inter-infusion interval (injections $p<0.001$, III $p<0.005$) (**Figure 6F**).

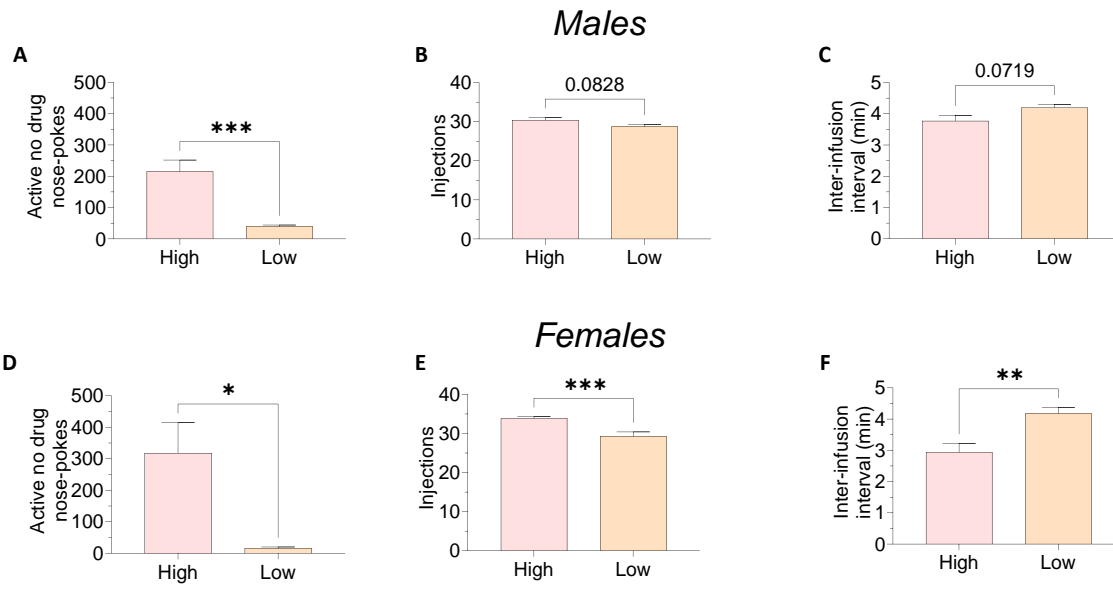


FIGURE 6: Manipulation 1: self-administration behavior in high and low maladaptive seekers – *Males*: High and low seekers differed in A. total active no drug nose-pokes, however, did not significantly differ in B. total injections or C. inter-infusion interval in the sessions used to characterize them as high or low seekers. *** $p < 0.001$
Females: High and low seekers differed in D. total active no drug nose-pokes, E. total injections, and F. inter-infusion interval in the sessions used to characterize them as high or low seekers. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Influence of cocaine maintenance (NCI) on maladaptive seeking (global)

Males

Non contingent injections of cocaine (NCI) during the first no drug period significantly reduced maladaptive seeking.

The active no drug nose-pokes in the NCI session was significantly lower than the average active no drug nose-pokes of three baseline sessions [N=62, ANOVA, condition effect: $F(1, 60)=4.1643$, $p=.04569$]. Considering both the NCI and the baseline sessions, there was a difference in active nose-pokes in the first five minutes compared to the last ten minutes of the no drug period [time effect: $F(1, 60)=33.038$, $p=.00000$] (**Figure 7A**). This time effect was a function of the condition tested however [condition x time effect: $F(1, 60)=9.2320$, $p=.00352$], attesting that the non-contingent cocaine injections during the no drug period leads to decreased maladaptive seeking behaviors. Expectedly, in the first five minutes of the no drug period (ie, the 5 minutes just prior to the first NCI), the

average number of active nose-pokes was equivalent in the baseline sessions and NCI session. Differently, in the last ten minutes of the period, the active nose-pokes in the NCI session was significantly lower than in the baseline sessions.

Females

The same observation was made as in the male experiment, ie non contingent injections of cocaine during the no drug period reduced maladaptive cocaine seeking.

The active no drug nose-pokes in the NCI session was significantly lower than the average active no drug nose-pokes of three baseline sessions [N=19, ANOVA, condition effect: $F(1, 17)=5.4777$, $p=.03171$]. In both the NCI sessions and the baseline sessions, there was a difference in active nose-pokes in the first five minutes compared to the last ten minutes of the no drug period [time effect: $F(1, 17)=11.025$, $p=.00405$] (**Figure 7B**).

This time effect was a function of the condition tested [condition x time effect: $F(1, 17)=14.545$, $p=.00139$].

While in the first five minutes of the no drug period, the average number of active nose-pokes was equivalent in the baseline sessions and NCI session (ie, the 5 minutes just prior to the first NCI), in the last ten minutes of the no drug period, the active nose-pokes in the NCI session was significantly lower than in the baseline sessions, highlighting that the non-contingent cocaine injections during the no drug period leads to decreased maladaptive seeking behaviors.

The NCI appeared to be more effective in females than in male rats, as in females active responding during the last 10 min did not anymore differ from the first 5 min.

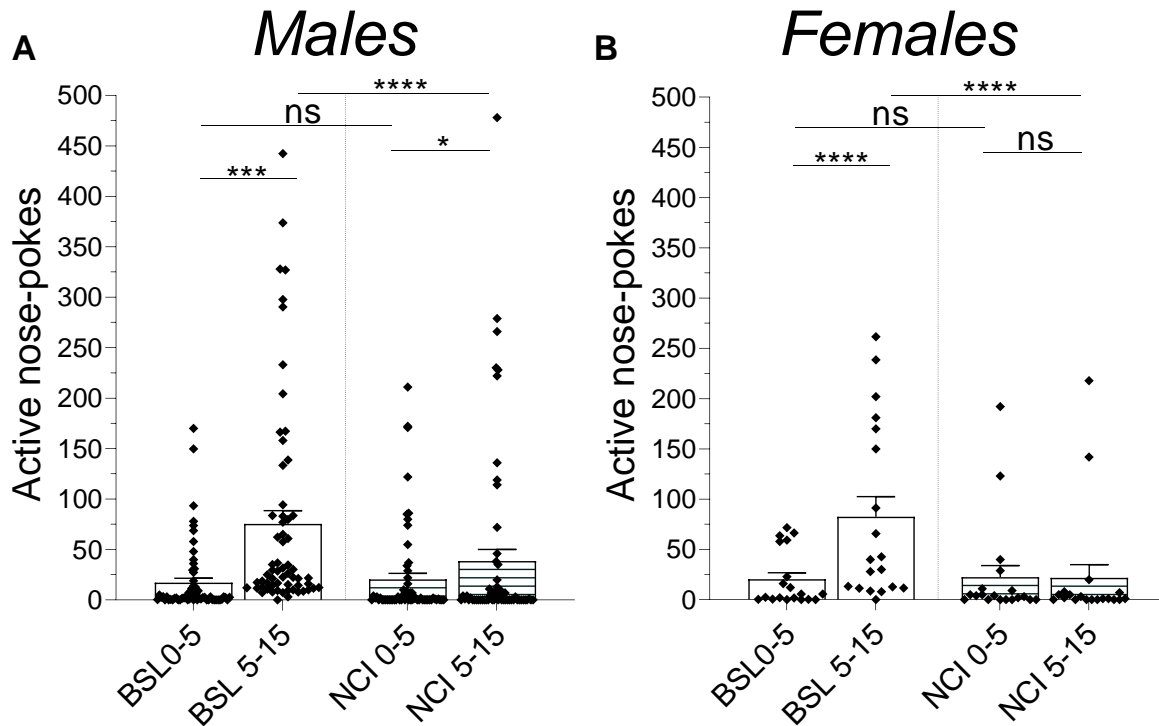


FIGURE 7: Manipulation 1: Non-contingent injection effect (global)- Males: A. In both baseline and NCI sessions, the first 5 minutes was significantly different from the last 10 minutes. The first 5 minutes of both baseline and NCI sessions did not differ. In the last 10 minutes, active nose-pokes decreased significantly between the basal and NCI conditions. **** $p < 0.0001$, *** $p < 0.001$, * $p < 0.05$

Females: B. The first 5 minutes was significantly different from the last 10 minutes of the baseline session, but not in the NCI session. The first 5 minutes of both baseline and NCI sessions did not differ. In the last 10 minutes, active nose-pokes decreased significantly between the basal and NCI conditions. **** $p < 0.0001$

Influence of cocaine maintenance (NCI) on maladaptive seeking as a function of vulnerability

Males

High seekers exhibited more active nose-pokes during no drug periods than low seekers [ANOVA, maladaptive group effect: $F(1, 60) = 17.139$, $p = .00011$] independent of the type of session (baseline vs NCI) [ANOVA, condition x maladaptive group effect: $F(1, 60) = .45466$, $p = .50272$], meaning that high seekers had higher no drug active nose-pokes than low seeker rats in both, the non-contingent injection session and the baseline session.

Precisely, the two groups differed in the trajectory of nose-poking [ANOVA, time x maladaptive group effect: $F(1, 60)=14.955, p=.00027$] with high rats showing higher active nose-pokes during the last 10 than during the first 5 min ($p<0.0001$), while no significant difference was observed for the low group ($p=0.2156$). The trajectory of active nose-pokes in the first five minutes and last ten minutes between groups was independent of the type of session [condition x maladaptive group x time effect: $F(1, 60)=2.1203, p=.15058$]. Therefore, the NCI produced a reduction in seeking independent of high or low groups (**Figure 8A and B**).

In summary, the effect of the non-contingent infusion was significant: decreasing maladaptive seeking patterns in the last ten minutes of the no drug period in both high and low seeker rats.

Females

High seekers exhibited more active nose-pokes during no drug periods than low seekers [ANOVA, maladaptive group effect: $F(1, 17)=7.1015, p=.01633$] independent of the type of session (baseline vs NCI) [ANOVA, condition x maladaptive group effect: $F(1, 17)=2.2470, p=.15222$], meaning that high seekers had higher no drug active nose-pokes than low seekers in both the non-contingent injection session and the baseline session.

Precisely, the two groups differed in the trajectory of nose-poking [ANOVA, time x maladaptive group effect: $F(1, 17)= 4.5434, p=.04794$] with high rats showing higher active nose-pokes during the last 10 than during the first 5 min ($p=0.0006$), while no significant difference was observed for the low group ($p=0.4454$).

The trajectory of active nose-pokes in the first five minutes and last ten minutes between groups differed as a function of the type of session [condition x maladaptive group x time effect: $F(1, 17)=6.9660, p=.01722$]. Importantly, in the high group, nose-pokes during the last 10 min of the NCI session were significantly lower than the corresponding 10 min in baseline ($p<0.0001$) (**Figure 8C**), while it was not significant in the low group ($p=0.27$) (**Figure 8D**).

In summary, the effect of the non-contingent infusion was significant: decreasing maladaptive seeking patterns in the last ten minutes of the no drug period. This effect depended on the group, significantly decreasing nose-poking in the high group while not reaching significance in the low group.

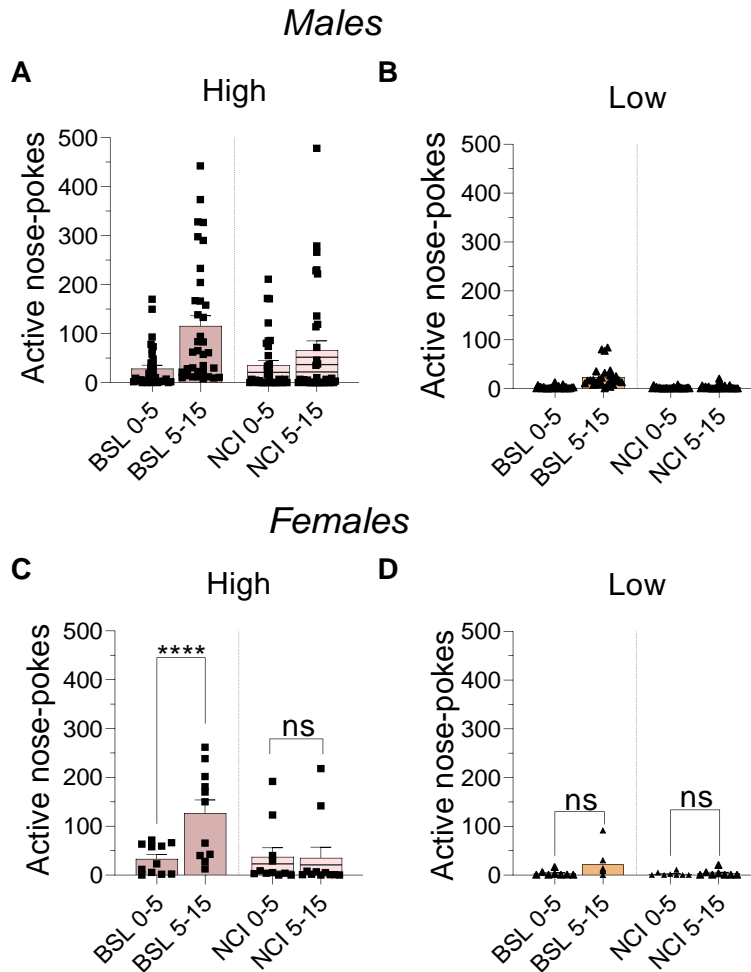


FIGURE 8: Manipulation 1: Non-contingent injection effect (high/low)- *Males*: A. There was a visible but non-specific decrease of no drug seeking in high rats. . B. There was a visible but non-specific decrease of no drug seeking in low rats. *Females*: C. In high maladaptive seekers, the first five minutes of the no drug period in the basal and NCI sessions were equivalent; however, the active nose-pokes in the last ten minutes of the no drug period between the basal and NCI sessions were significantly different showing that the non-contingent injection successfully decreased maladaptive seeking patterns. D. In low maladaptive seekers, the first 5 minutes during the no drug period in the basal and NCI sessions were also equivalent. However, unlike the high rats, there was not a significant difference between the last ten minutes of the basal and NCI sessions. Non-contingent injections significantly affect maladaptive seeking behavior in high rats while this effect is not as significant in low seekers. **** $p < 0.0001$

Manipulation 2: Effect of extending the duration of the no drug period session on initiation of maladaptive seeking

Acquisition of self-administration behavior

Rats acquired cocaine self-administration as shown by both the significant discrimination between the active and inactive holes [ANOVA, hole effect: $F(1, 23)=94.142$, $p<0.0001$] increasing over sessions [hole x session effect: $F(15, 345)=6.4115$, $p<0.0001$] and an increase in the number of cocaine injections per session which achieved stability in the population by day 10 [N=25, ANOVA, session effect: $F(15, 345)=18.595$, $p<0.0001$] [Fisher LSD post-hoc].

Regarding no drug periods, we analyzed here the first one (as this is the one that will be used for rats' classification as high and low maladaptive seekers). In this period, a higher number of responding in the active hole was also observed [ANOVA, hole effect: $F(1, 21)=6.2384$, $p<0.05$] however, fluctuation did not occur over the sessions [hole x session effect: $F(15, 315)=1.2565$, $p=0.2287$].

Identification and classification of maladaptive seeking groups

Manipulation 2 was performed on a single cohort. Classification of high and low seekers was done on the average no drug responses on days 14-16.

In the drug period, the evolution of responding (hole x session effect) previously observed was not a function of vulnerability [ANOVA (Drug): hole x session x maladaptive group effect: $F(15, 345)=1.2745$, $p=0.2158$].

In the no drug period, progression of discrimination over the acquisition phase did not significantly differ between high and low seekers [No drug 1: hole x session x maladaptive group effect: $F(15,315)=1.242$, $p=0.24$]. Although non-significant, the profile was very similar to the one observed for the NCI males, with high seeker rats starting showing higher level of active nose-pokes than low seeker rats, starting session 8 (data not shown).

Mean injections per session [ANOVA, maladaptive group effect: $F(1, 23)=1.4746$, $p=0.24$] nor the trajectory of injections over the acquisition period differed as a function of group [ANOVA, session x maladaptive group effect: $F(15, 345)=1.4569$, $p=0.12$].

As expected, over the sessions used to characterize high and low seekers, average total active no drug nose-pokes were significantly different between the high (n=15) and low (n=10) seeker groups ($p < 0.001$) (**Figure 9A**). The number of average total injections (**Figure 9B**) and average inter-infusion interval over these sessions did not significantly differ between groups, however (**Figure 9C**) (injections $p = 0.1933$, III $p = 0.4382$).

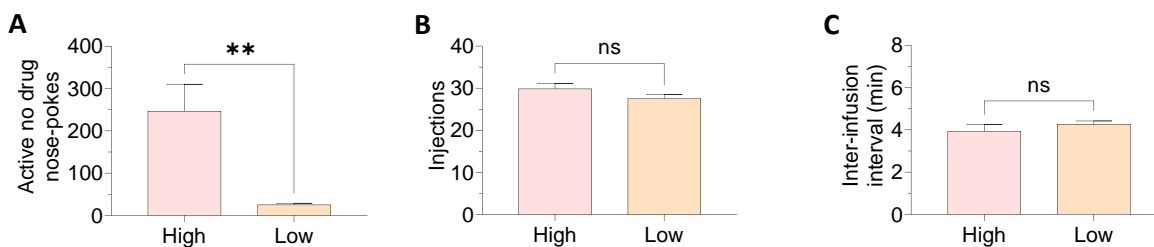


FIGURE 9: Manipulation 2: self-administration behavior in high and low maladaptive seekers- High and low seekers differed in A. total active no drug nose-pokes, but not in B. total injections or C. inter-infusion interval during the sessions used to characterize them as high or low. ** $p < 0.01$

Effect of long ND period as a function of vulnerability

The long ND period was split up into bins of 15 minutes. There was an effect of time on active nose-poke seeking during the long no drug period [time effect: $F(5, 115) = 5.2105$, $p = .00024$]. Throughout the long ND, there was a significant difference in seeking behaviors between high and low seekers [ANOVA, maladaptive group effect: $F(1, 23) = 7.7744$, $p = .01045$] and the two groups had a different trajectory of responding through the long no drug period [maladaptive group x time effect: $F(5, 115) = 4.2923$, $p = .00129$]. More specifically, the low seekers did not express any changes in seeking patterns throughout the entire long ND period: their behavior was stable and low throughout the entire long no drug period. However, in the high seekers, the high pattern of responding observed in the first 15 min (corresponding to a classical ND period duration) prolonged over the next 15 minutes. The four last bins (between 30-90 minutes) did not significantly differ between high and low seekers, indicating that the high seekers significantly decreased responding after the first 30 minutes despite cocaine concentration remained low.

Implementation of a long no drug period showed that while high seekers express high seeking patterns in the first 30 minutes, low no drug seekers consistently withhold seeking patterns with a continuous illumination of a discriminative stimulus signaling drug unavailability (**Figure 10**). This adds to the previous evidence showing that, contrary to high seekers, low seekers exhibit controlled seeking that is sensitive to external discriminative stimuli. Also despite the fact that cocaine concentration did not re-increase, high seekers showed a progressive decrease in maladaptive seeking after 30 minutes, indicating a possible extinction effect and loss of behavioral control by internal state after a given duration.

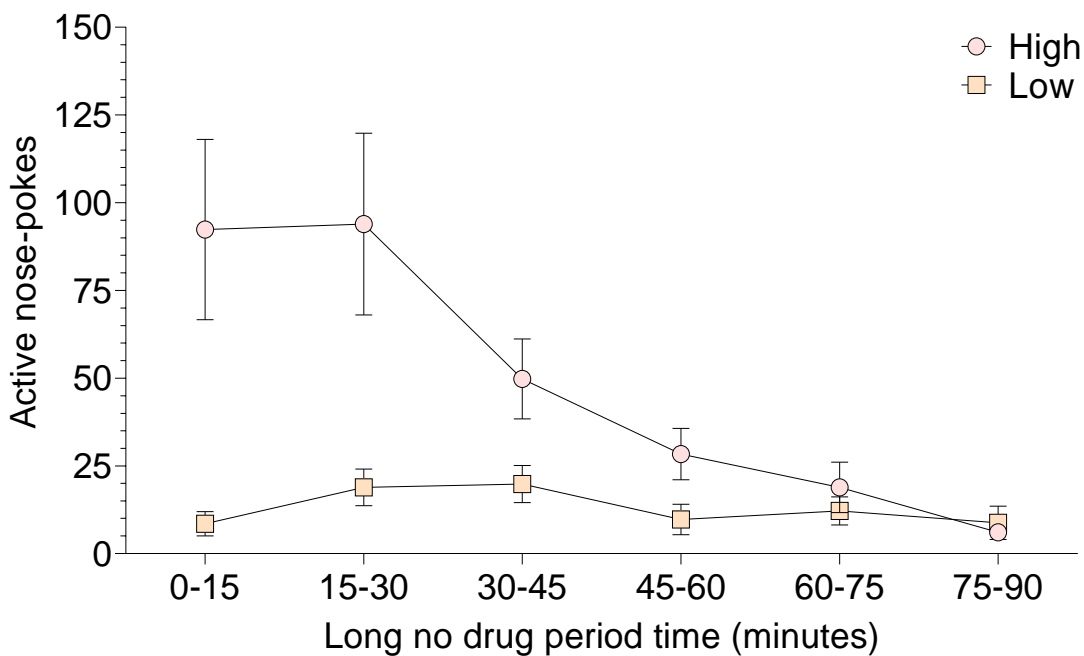


FIGURE 10: Manipulation 2: Long no drug period sessions (high/low)- The long no drug period seeking patterns differed in high and low seekers. High seekers had strong seeking patterns in the beginning of the long no drug period, whereas low seekers had low seeking patterns throughout the whole long no drug period, with no significant difference between the six 15-minute bins.

Manipulation 3: Effect of a drug free state on maladaptive seeking expression

Acquisition of self-administration behavior

Males

Rats acquired cocaine self-administration as shown by both the significant discrimination between the active and inactive holes [ANOVA, hole effect: $F(1, 22)=98.253$, $p<0.0001$] increasing over sessions [hole x session effect: $F(15, 330)=7.2442$, $p<0.0001$] and an increase in the number of cocaine injections per session which achieved stability in the population by day 10 [N=24, ANOVA, session effect: $F(15, 330)=22.595$, $p<0.0001$].

Regarding no drug periods, we analyzed here the first one (as this is the one that will be used for rats' classification as high and low maladaptive seekers). In this period, a higher number of responding in the active hole was also observed [ANOVA, hole effect: $F(1, 19)=9.9291$, $p<0.01$], however significant fluctuation did not occur over the sessions though there was a strong trend [hole x session effect: $F(15, 285)=1.6941$, $p=0.0514$].

Females

Rats acquired cocaine self-administration as shown by both the significant discrimination between the active and inactive holes [ANOVA, hole effect: $F(1, 17)=57.897$, $p<0.0001$] increasing over sessions [hole x session effect: $F(16, 272)=3.9300$, $p<0.0001$] and an increase in the number of cocaine injections per session which achieved stability in the population by day 7 [N=19, ANOVA, session effect: $F(16, 272)=28.326$, $p<0.0001$] [Fisher LSD post-hoc].

Regarding no drug periods, we analyzed here the first one (as this is the one that will be used for rats' classification as high and low maladaptive seekers). In this period, a higher number of responding in the active hole was also observed [ANOVA, hole effect: $F(1, 14)=14.117$, $p<0.005$] with fluctuation over the sessions [hole x session effect: $F(16, 224)=1.6906$, $p<0.05$].

Identification and classification of maladaptive seeking groups

Males

Manipulation 3 (ND pre) was done on a single cohort, therefore establishment of high and low maladaptive seekers was based on the average no drug seeking patterns in the intoxicated no drug period (ND1) of three ND pre sessions. The average ND1 responses were averaged over the second to fourth ND pre sessions, then a median split determined the high and low seekers.

In the drug period, the evolution of responding (hole x session effect) previously observed was not a function of vulnerability, ie similar in high and low no drug seekers [Drug: hole x session x maladaptive group effect: $F(15, 330)=1.3990$, $p=0.14529$].

Though there was trend in the evolution of responding in the no drug period (hole x session effect), this was not a function of vulnerability [No drug 1: hole x session x maladaptive group effect: $F(15, 285)=1.3862$, $p=0.15278$]. Although non-significant, the profile was very similar to the one observed for the NCI males, with high seeker rats starting showing higher level of active nose-pokes than low seeker rats, starting session 8 (data not shown).

Additionally, although the pattern of injections over time did not differ between the two groups [ANOVA, maladaptive group x session effect: $F(15, 330)=0.62603$, $p=0.85338$], the two groups differed for the total number of injections over the acquisition period [ANOVA, maladaptive group effect: $F(1, 22)=12.770$, $p<0.005$].

Between the high ($n=12$) and low ($n=12$) groups, on the sessions used to identify them, the average total active no drug nose-pokes were significantly different ($p<0.0001$) (**Figure 11A**) as expected. However, high or low seekers also differed for the average number of total injections (**Figure 11B**) and the average inter-infusion interval on these sessions (injections: $p<0.01$, III: $p<0.001$) (**Figure 11C**).

Females

Manipulation 3 (ND pre) was done on a single cohort, therefore establishment of high and low maladaptive seekers was based on the average no drug seeking patterns in the intoxicated no drug period (ND1) of three ND pre sessions. The average ND1 responses

were averaged over the second to fourth ND pre sessions, then a median split determined the high and low seekers.

In the drug period, the evolution of responding (hole x session effect) previously observed was not a function of vulnerability [Drug: hole x session x maladaptive group effect: $F(16, 272)=0.89226$, $p=0.57869$]. Neither the total injections [ANOVA, maladaptive group effect: $F(1, 17)=2.8108$, $p=.11192$] nor the time course of injections over the acquisition period differed between the two groups [ANOVA, session x maladaptive group effect: $F(16, 272)=0.8633$, $p=0.61236$].

Additionally, the evolution in responding in the no drug period did not differ between high and low rats [No drug 1: hole x session x maladaptive group effect: $F(16, 224)=0.95854$, $p=0.50333$]. The profile was different from the one observed earlier, with high seeker rats starting to show a higher level of active nose-pokes starting at session 15 (data not shown).

Between the high ($n=9$) and low ($n=10$) groups, the average total active no drug nose-pokes were significantly different ($p= 0.001325$) (**Figure 11D**) as expected, but not the average number of total injections (**Figure 11E**) or the average inter-infusion interval of the sessions on the sessions used to identify them as high or low seekers despite the presence of trends (injections $p=0.05562$, III $p= 0.085223$) (**Figure 11F**).

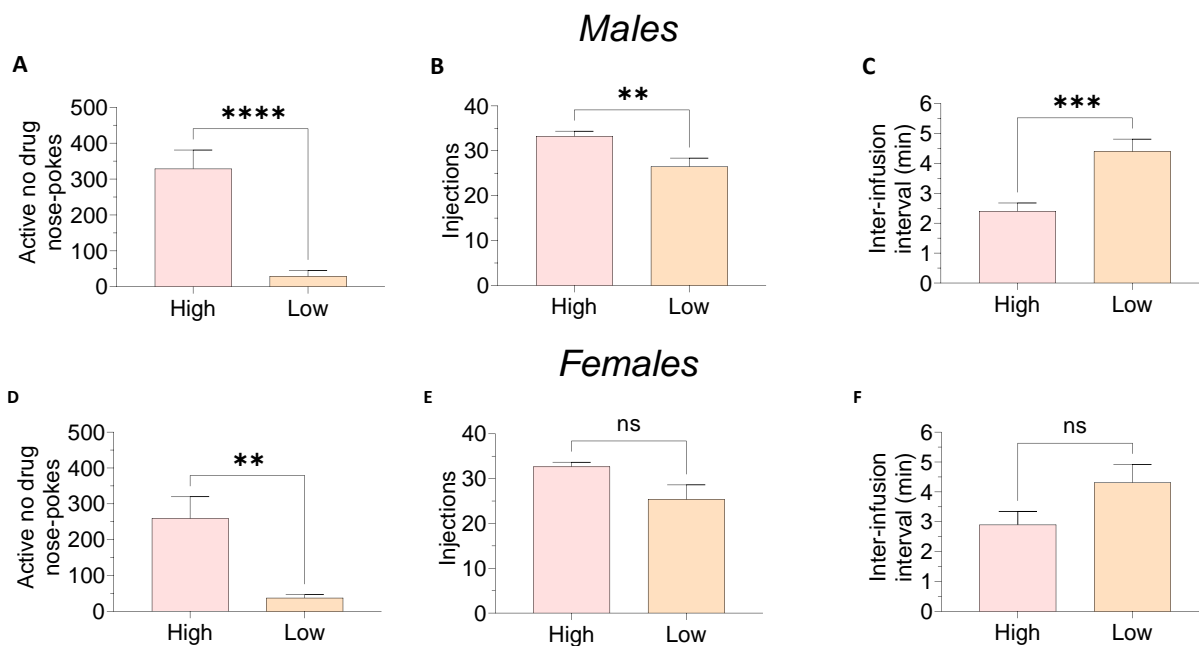


FIGURE 11: Manipulation 3: self-administration behavior in high and low maladaptive seekers- *Males*: High and low seekers differed in A. total active nose-pokes, B. total injections, and C. inter-infusion interval during the sessions used to characterize them as high or low. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$

Females: High and low seekers differed in D. total active nose-pokes, but not total injections, nor F. inter-infusion interval during the sessions used to characterize them as high or low. ** $p < 0.01$

Relationship between maladaptive seeking expression and cocaine status (global)

Males

In all rats, a significant difference in active nose-pokes was observed between no drug periods in a drug free state compared to no drug periods in which the individuals were under cocaine effect [ANOVA, condition effect: $F(1, 22) = 13.822$, $p = .00120$] (**Figure 12A**).

Females

In all rats, there was a significant difference in active nose-pokes between no drug periods in a drug free state compared to no drug periods in which the individuals were under cocaine effect [ANOVA, condition effect: $F(1, 17) = 4.6419$, $p = .0458$] (**Figure 12B**).

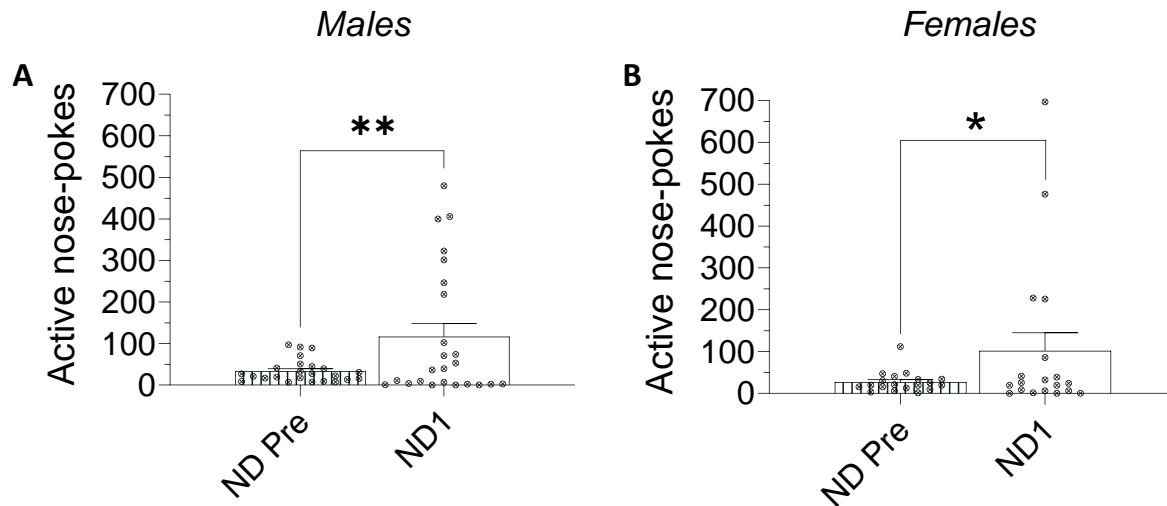


FIGURE 12: Manipulation 3: No drug pre (global)- Males: A. Active nose-pokes between the drug free no drug period and the intoxicated no drug period significantly differed at the population level $**p<0.01$
Females: B. Active nose-pokes between the drug free no drug period and the intoxicated no drug period significantly differed at the population level $*p<0.05$

Relationship between maladaptive seeking expression and cocaine status as a function of vulnerability

Males

As shown previously, maladaptive responding was significantly higher in the intoxicated state (ND1) than in the drug free state (ND pre) at the population level. The difference between high and low groups was depending on the drug state however [ANOVA, maladaptive group effect: $F(1, 22)=28.170$, $p=.00003$; maladaptive group x condition effect: $F(1, 22)=17.478$, $p=.00039$]. Post hoc analysis on the maladaptive group x condition effect [Fisher LSD post hoc] indeed revealed that the difference between high and low rats in responding during the intoxicated state, on which they are classified ($p<0.0001$), is no more present in the drug free ND period (ND pre) ($p=0.301015$) (**Figure 13A**).

In addition, in the low group, active nose-pokes during the no drug pre period did not differ from the active nose-pokes during the intoxicated no drug period ($p=0.746549$). Differently, high rats produced more active nose-pokes during the intoxicated ND period than during the drug free ND period ($p<0.0001$).

In conclusion, seeking patterns in high maladaptive seekers (which normally express maladaptive seeking in the no drug periods when under cocaine effect) do not significantly differ from low rats when in a drug free state. In addition, low rats are not significantly affected by cocaine: their no drug seeking is comparable whether they are intoxicated or not.

Females

As shown previously, maladaptive responding was significantly higher in the intoxicated state (ND1) than in the drug free state (ND pre) at the population level. The difference between high and low groups was depending on the drug state however [ANOVA, maladaptive group effect: $F(1, 17)=7.6434$, $p=0.01325$; maladaptive group x condition effect: $F(1, 17)=6.0420$, $p=0.025$]. Post hoc analysis on the condition x maladaptive group effect [Fisher LSD post hoc] revealed that the difference between high and low rats in responding during the intoxicated state, on which they are classified ($p=0.0013$), is no more present in the drug free ND period (ND pre) ($p=0.7701$) (**Figure 13B**).

In addition, in the low group, active nose-pokes during the no drug pre period did not differ from the active nose-pokes during the intoxicated no drug period ($p=0.82808$). Differently, high rats produced more active nose-pokes during the intoxicated ND period than during the drug free ND period ($p=0.00549$).

In conclusion, in both male and female, the drug status had the same impact on no drug responding. Maladaptive seeking was depending on the drug status, ie high seekers expressed maladaptive seeking under cocaine effect only (ND1). Low seekers expressed low drug seeking in no drug periods independent of the drug status.

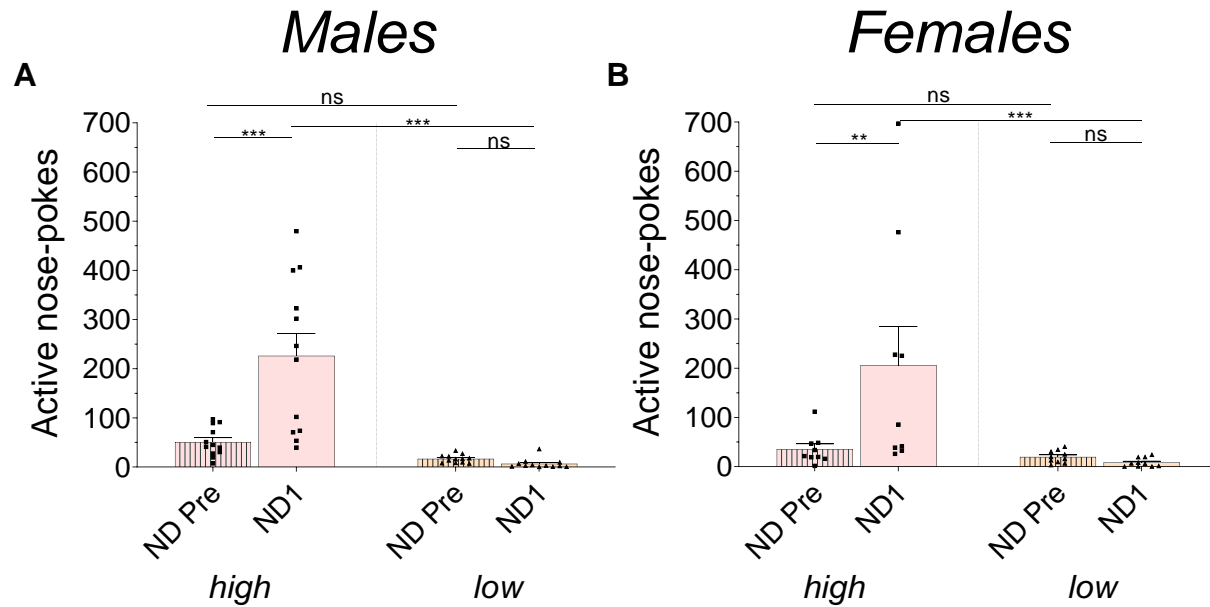


FIGURE 13: Manipulation 3: No drug pre (high/low)- Males: A. No drug pre active nose-pokes did not significantly differ between high and low seekers. Additionally, in low seekers, no drug seeking was not strongly influenced by cocaine- it remained equivalent between drug free and intoxicated states. However, in high seekers, there was a significant increase in active nose-pokes from a drug free no drug period to an intoxicated no drug state. *** $p < 0.001$

Females: B. No drug pre active nose-pokes did not significantly differ between high and low seekers. Additionally, in low seekers, no drug seeking was not strongly influenced by cocaine- it remained equivalent between drug free and intoxicated states. However, in high seekers, there was a significant increase in active nose-pokes from a drug free no drug period to an intoxicated no drug state. *** $p < 0.001$ ** $p < 0.01$

ADAPTIVE INHIBITION OVER INCENTED COCAINE SEEKING

Manipulation 4: Effect of adaptive inhibitory discriminative stimulus (no drug DS) on cocaine-induced reinstatement

Acquisition of self-administration behavior

Males

Rats acquired cocaine self-administration as shown by both the significant discrimination between the active and inactive holes [ANOVA, hole effect: $F(1, 82)=112.90$, $p < 0.0001$] increasing over sessions [hole x session effect: $F(16, 1312)=10.040$, $p < 0.0001$] and an increase in the number of cocaine injections per session which achieved stability in the

population by day 11 [N=86, ANOVA, session effect: $F(16, 1344)=56.198$, $p<0.00001$] [Fisher LSD post-hoc].

Regarding no drug periods, we analyzed here the first one (as this is the one that will be used for rats' classification as high and low maladaptive seekers). In this period, a higher number of responding in the active hole was also observed [ANOVA, hole effect: $F(1, 70)=53.785$, $p<0.0001$] with fluctuation over the sessions [hole x session effect: $F(16, 1120)=4.4322$, $p<0.0001$].

Females

Rats acquired cocaine self-administration as shown by both the significant discrimination between the active and inactive holes [ANOVA, hole effect: $F(1, 10)=24.700$, $p=0.0006$] increasing over sessions [hole x session effect: $F(16, 160)=2.8117$, $p=0.0005$] and an increase in the number of cocaine injections per session which achieved stability in the population by day 7 [N=14, ANOVA, session effect: $F(16, 160)=26.975$, $p<0.001$] [Fisher LSD post-hoc].

Regarding no drug periods, we analyzed here the first one (as this is the one that will be used for rats' classification as high and low maladaptive seekers). In this period, a higher number of responding in the active hole was also observed [ANOVA, hole effect: $F(1, 10)=8.9488$, $p<0.03$] however, unlike males, fluctuation did not occur over the sessions [hole x session effect: $F(16, 160)=0.55897$, $p=0.971016$].

Identification and classification of maladaptive seeking groups

Males

Manipulation 4 (reinstatement) was performed on multiple cohorts. Therefore, classification of high and low seekers was done on the average no drug responses on days 15-17 before protocol differences occurred in the experiments.

High and low seekers from the two DS groups did not differ for responding and discrimination during the acquisition phase [Drug: hole x DS group x maladaptive group effect: $F(1,82)=0.88$, $p=0.35$].

Additionally, as expected, the evolution in responding (hole x session effect) in the no drug period was a function of vulnerability [No drug 1: hole x session x maladaptive group effect: $F(16, 1120)=2.7493$, $p<0.0005$] indicative of a progressive development of

maladaptive seeking in high rats. Notably this evolution was a function of the DS group [No drug 1: hole x session x maladaptive group x DS group effect: $F(16, 1120)=2.2757$, $p<0.005$] due to High DDS rats having less stability in active no drug responding from session 12 to 17 than High ND DS rats (data not shown).

Additionally, though acquisition of injections differed between groups [ANOVA, maladaptive group effect: $F(1, 84)=14.321$, $p<.0005$], both low and high rats exhibited similar injection acquisition patterns over time [ANOVA, maladaptive group x session effect: $F(16, 1344)=.43510$, $p=.97365$].

As expected, over the sessions used to characterize them the high ($n=40$) and low ($n=46$) seeker groups differed for the average total active no drug nose-pokes ($p< 0.0001$) (**Figure 14A**). They also differed for the number of average total injections (**Figure 14B**) and average inter-infusion interval over these sessions (**Figure 14C**) (injections $p< 0.01$, III $p< 0.01$).

Females

Manipulation 4 was performed on a single cohort. Classification of high and low seekers was done on the average no drug responses on days 15-17.

In the drug period, the evolution of responding (hole x session effect) previously observed did not differ as a function of vulnerability [ANOVA (Drug): hole x session x maladaptive group effect: $F(16, 160)=0.82915$, $p=0.65115$].

Additionally, both injections [ANOVA, maladaptive group effect: $F(1, 10)=5.0629$, $p<0.05$], and injection patterns over time differed as a function of group [ANOVA, maladaptive group x session effect: $F(16, 160)=2.1742$, $p<0.01$].

Between the high ($n=9$) and low ($n=5$) groups, average total active no drug nose-pokes did not significantly differ ($p= 0.109484$) (**Figure 14D**). They did not differ in the number of average total injections ($p=0.203647$) (**Figure 14E**), nor the average inter-infusion interval over the sessions used to characterize them (**Figure 14F**) ($p= 0.29166$).

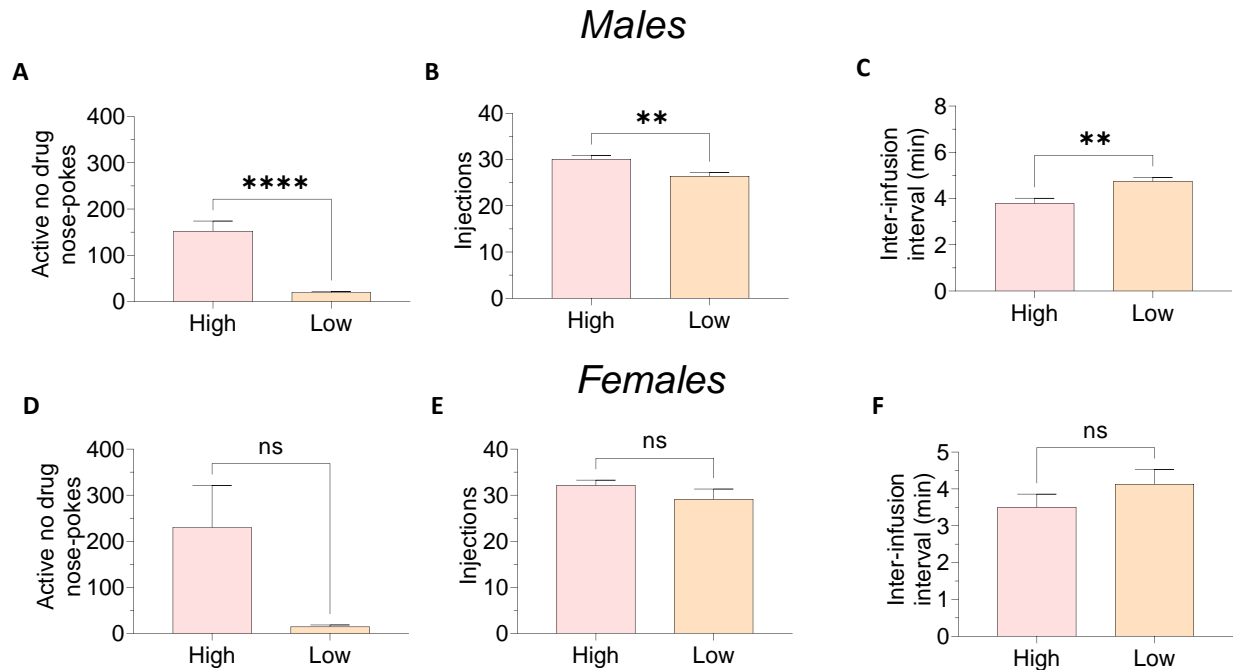


FIGURE 14: Manipulation 4: self-administration behavior in high and low maladaptive seekers- *Males*: High and low seekers differed in A. total active nose-pokes, B. total injections, and C. inter-infusion interval during the sessions used to characterize them as high or low. **** $p < 0.0001$, ** $p < 0.01$
Females: High and low seekers did not significantly differ in D. total active nose-pokes, E. total injections, or F. inter-infusion interval during the sessions used to characterize them as high or low.

Cocaine-induced reinstatement under drug and no drug discriminative stimulus (global)

Males

During extinction, rats significantly discriminated between the active and inactive holes [ANOVA, hole effect: $F(1, 82) = 162.85, p = 0.0000$] independent of if the reinstatement session would be under drug or no drug discriminate stimuli [hole x DS group effect: $F(1, 82) = .17786, p = .67433$]. There was no difference between the drug DS and no drug DS group in terms of total responding during extinction as well as in discrimination between active and inactive holes.

Responding decreased over the extinction phase [N=86, ANOVA, time effect: $F(59, 4838) = 14.115, p < 0.0001$], which was due to a decrease in responding patterns towards

the end of the extinction period- resulting in no significant difference between active and inactive hole at the end [hole x time effect: $F(59,4838)=7.71795$, $p<0.0001$].

Total nose-pokes over the reinstatement phase differed according to the DS presented (Drug DS $n=43$, No drug DS $n=43$) [$N=86$, ANOVA, DS group effect: $F(1, 82)=14.513$, $p=.00027$], with the No drug DS condition generating less nose-pokes. This effect of DS condition was depending on the cocaine dose [dose x DS group effect: $F(3, 246)=6.2830$, $p=.00040$]. Active nose-pokes were specifically concerned by this effect of DS as a function of cocaine dose [hole x dose x DS group effect: $F(3, 246)=5.0500$, $p=.00206$]. Active nose-pokes were indeed significantly lower in the ND DS condition than in the drug DS group at doses 0.8 mg/kg/inf ($p<0.00001$) and 1.6 mg/kg/inf ($p<0.00001$) (**Figure 15A**). The D and ND DS groups did not differ for inactive nose-pokes at any dose.

In summary, at the population level, the ND DS was able to significantly reduce reinstatement. Under D DS, cocaine induced a significant reinstatement starting dose 0.4 mg/kg, as measured through a significant discrimination between active and inactive nose-poking (Fisher LSD post-hoc, $p<0.05$ for dose 0.4, $p<0.0001$ for doses 0.8 and 1.6). Under ND DS, cocaine induced reinstatement only at the two highest doses ($p<0.05$ for dose 0.8 and $p<0.0001$ at dose 1.6) and of much lower amplitude as measured through active nose-poking ($p<0.00001$) in absence of differences in inactive nose-poking.

Females

During extinction, rats significantly discriminated between the active and inactive holes [ANOVA, hole effect: $F(1, 10)= 69.649$, $p<0.0001$] independent of if the reinstatement session would be under drug or no drug discriminate stimuli [hole x DS group effect: $F(1, 10)=2.1069$, $p=0.17727$]. There was no difference between the drug DS and no drug DS group in terms of total responding during extinction as well as in discrimination between active and inactive holes.

Responding decreased over the extinction phase [$N=14$, ANOVA, time effect: $F(59, 590)=3.6053$, $p<0.0001$], which was due to a decrease in responding patterns towards the end of the extinction period- resulting in no significant difference between active and inactive hole at the end [hole x time effect: $F(59,590)=2.6966$, $p<0.0001$].

Cocaine induced a significant reinstatement as attested by a higher number of active over inactive nose-poking [Hole effect, $F(1,12)=21.53$, $p<0.001$]. Total nose-pokes over the reinstatement phase differed according to the DS presented (Drug DS vs No drug DS) [N=14, ANOVA, DS group effect: $F(1,12)=6.08$, $p<0.05$], with the No drug DS condition generating less nose-pokes. This effect of DS condition was not depending on the cocaine dose [dose x DS group effect: $F(3, 36)=1.98$, $p=0.13$] nor the hole [Hole x DS effect: $F(1,12)=0.008$, $p=0.93$], however (**Figure 15B**).

In summary, at the population level, the ND DS was able to significantly reduce responding globally at all doses but nonspecifically.

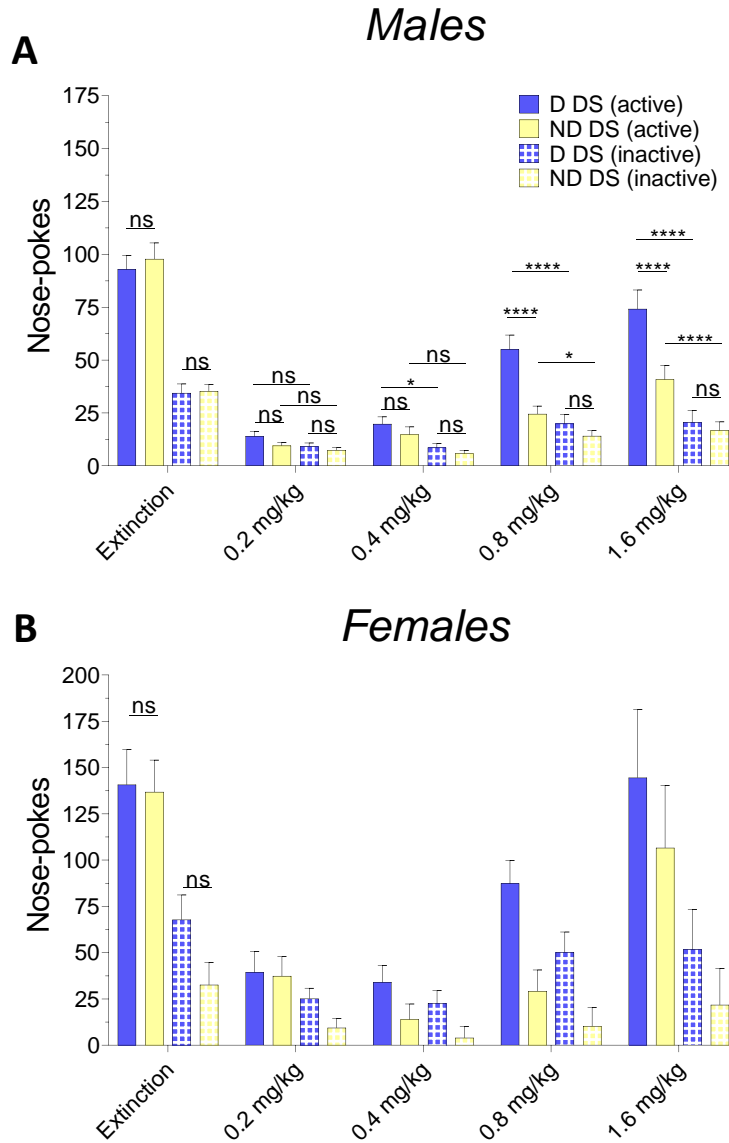


FIGURE 15: Manipulation 4: cocaine-induced reinstatement under drug and no drug DS-
Males: A. Between the drug and no drug DS groups, extinction was equivalent. ND DS decreased cocaine-induced reinstatement with a specific effect on active nose-poking for the two highest doses administered **** $p < 0.0001$, * $p < 0.05$

Females: B. Between the drug and no drug DS groups, extinction was equivalent. Global responding in cocaine-induced reinstatement was higher in the drug DS group than it was for the no drug DS group.

Relationship between maladaptive seeking and cocaine-induced reinstatement under drug and no drug discriminative stimulus

Males

During extinction the four groups discriminated significantly between active and inactive holes (high D DS n=17, low D DS n=26, high ND DS n=23, low ND DS n=20) [Hole effect, $F(1,82)=162.85$, $p<0.0001$]. Despite an apparent difference in discrimination in high and low seekers as a function of the DS condition [hole x DS group x maladaptive group interaction [$F(1, 82)=5.4780$, $p<0.05$], the four groups did not differ for the total number of active nose pokes or the total number of inactive nose-pokes over the extinction phase [DS group x maladaptive group, $F(1,82)=0.93$, $p=0.33$]. They also all significantly discriminated between active and inactive holes (high D DS $p<0.001$, high ND DS $p<0.001$, low D DS $p<0.001$, low ND DS $p<0.001$).

Responding in extinction decreased over time with a similar time course in the high and low seeker groups [time x maladaptive group effect: $F(59,4838)=0.77762$, $p=0.89303$] as well as if the reinstatement would be under D or ND DS [time x DS group effect: $F(59,4838)=1.0172$, $p=0.43935$].

During the whole cocaine-induced reinstatement session, low seeker and high seekers had significantly different combined active and inactive nose-poking activity [maladaptive group effect: $F(1, 82)=7.9420$, $p<0.01$] regardless of the discriminative stimulus present [maladaptive group effect x DS group: $F(1, 82)=.01049$, $p=.91869$]. The difference in nose-poking patterns of the high and low groups was function of the dose administered, however [maladaptive group x dose effect: $F(3, 246)=5.0356$, $p=.00210$].

The pattern of responding between active and inactive holes was depending on the dose of cocaine [hole x dose effect: $F(1,3)=21.88$, $p<0.00001$], but this dose dependence was similar for high and low seekers [hole x dose x maladaptive group effect: $F(3, 246)=1.2758$, $p=.28322$].

Notably, under D DS, cocaine induced reinstatement (as measured through significant difference between active and inactive nose-pokes) started at dose 0.4 mg/kg in the high rats (**Figure 16A**) and at 0.8 mg/kg in the low rats (**Figure 16B**). Under ND DS, cocaine induced reinstatement occurred at both 0.8 mg/kg and 1.6 mg/kg in the high rats (**Figure 16A**), but only at 1.6 mg/kg in the low rats (**Figure 16B**). The cocaine training dose lost the ability to induce reinstatement in the low seekers under ND DS.

Males

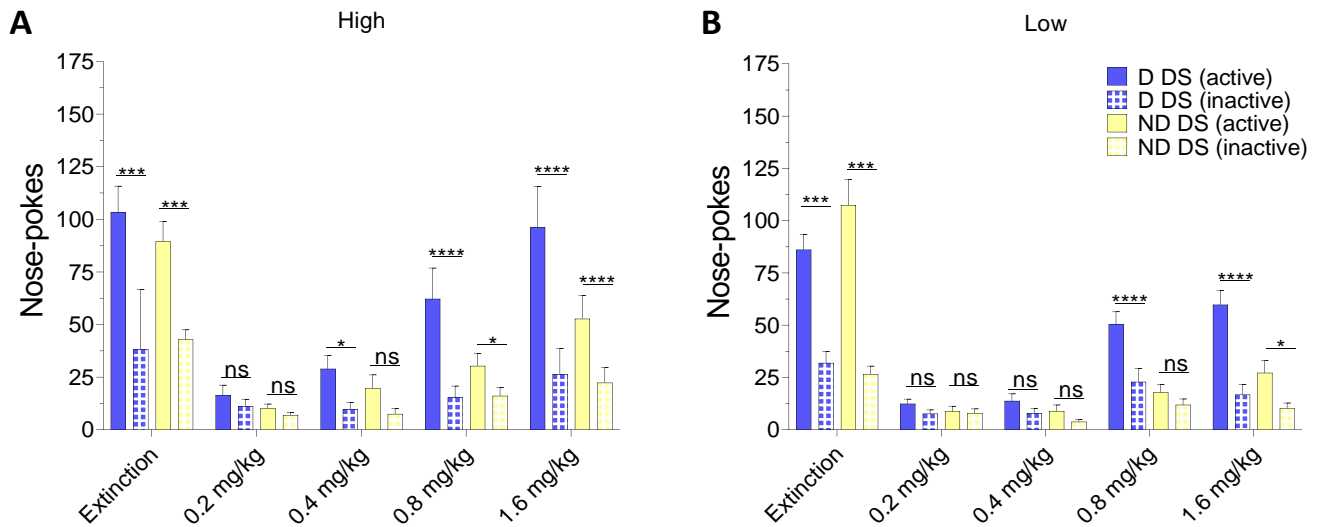


FIGURE 16: Manipulation 4: cocaine-induced reinstatement (active and inactive holes)

under drug and no drug DS as a function of vulnerability- Males: A. Active and inactive responding in high rats under both D DS and ND DS. During extinction and at 0.8/1.6 mg/kg/infusion, high rats in both DS conditions discriminated between the active and inactive holes. B. Active and inactive responding in low rats under both D DS and ND DS. During extinction and at 1.6 mg/kg/infusion, rats in both D DS and ND DS conditions discriminated between the active and inactive holes. However, at 0.8 mg/kg/infusion, only the D DS group had a significant difference in active and inactive responses, due to the low levels of active hole responding in the low ND DS group. **** $p < 0.00001$, *** $p < 0.001$, * $p < 0.05$ as compared to inactive nose-pokes.

At the highest dose (1.6 mg/kg/infusion), the high seekers had significantly different active nose-pokes in both the drug DS ($p < 0.005$) and no drug DS groups ($p < 0.0005$) compared to the low seekers (**Figure 17**).

Males

Cocaine-induced reinstatement

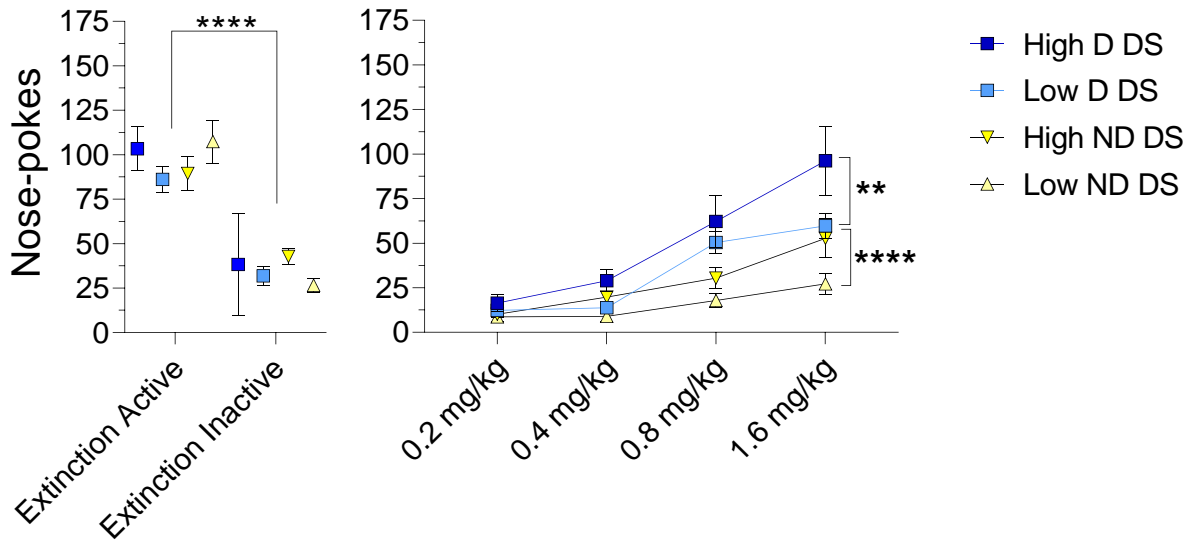


FIGURE 17: Manipulation 4: cocaine-induced reinstatement (active hole) under drug and no drug DS as a function of vulnerability (high/low)- Males: Whether in D DS or ND DS condition, high and low seekers did not differ in extinction. However, at the highest dose in reinstatement, the high seekers had significantly higher active nose-poking activity compared to the low seekers in both the drug DS and the ND DS condition. **** $p < 0.0001$, ** $p < 0.01$, as compared to low seekers.

DISCUSSION

Maladaptive seeking expression seems to be a result of a higher sensitivity to changes in internal state.

Adaptive inhibition as a function of drug state

The maladaptive seeking behavior that is characteristic of future addicted rats was hypothesized to be due to internal signals telling the rat that it is no longer at a level of ideal intoxication and to proceed with drug seeking behaviors despite the environment signaling drug unavailability. Injecting non-contingent and non-signaled cocaine during the no drug period prevented these maladaptive seeking behaviors from emerging, alluding to the fact that drug craving is a crucial factor contributing to these behaviors.

The effect of the non-contingent injection was significant in the high seekers while failing to reach significance in the low seekers. The effect of the non-contingent injection was identical in both sexes.

As the reason for maladaptive seeking observed in the high seekers is believed to be due to less-than ideal intoxication levels, it was questioned if this threshold for reinitiation of seeking existed in low seekers, but the duration of the normal 15-minute no drug period was too short to observe it. If this was the case, then low seekers would express a reinitiation if the no drug period was longer. We therefore extended the duration of the no drug period to 90 minutes. However, as this reinitiation was not observed, it is believed that the low seekers have higher adaptive inhibitory control that is mediated by the environmental stimuli. While the high seekers' adaptive behavioral control seems to be more influenced by their internal state, high seeking did not persist all over the duration of the long no drug period. It persisted over 30 minutes (ie an additional 15 min to the basal 15 min no drug period) but decreased progressively suggesting that seeking driven by decreases in cocaine concentration was sensitive to extinction processes.

Cocaine maintenance influences expression of maladaptive seeking in a cocaine intoxicated state, leading to questions regarding the significance of the inhibitory DS in the high and low seekers. The addition of a no drug period to beginning of the baseline self-administration session allowed for a novel behavior to be observed: seeking behavior during a no drug period in a drug-free state. Critically, maladaptive behavior was expressed significantly less in the drug-free no drug period than in the intoxicated no drug period in both males and females. The reduction of active hole nose-pokes between the drug-free no drug period and intoxicated no drug period was significant in the maladaptive high seekers while not reaching significance in the low seekers.

These results show that in high no drug seekers, at this moment before the transition to addiction occurs, the no drug inhibitory discriminative stimulus still can be integrated into guiding behavior as shown by the ability of vulnerable rats to decrease maladaptive seeking behavior in a drug-free no drug period. Consequently, the maladaptive seeking patterns that are characteristic of vulnerable rats are not simply due to a lack of incorporation of the discriminative stimulus, as the significance of the discriminative is learned: evidenced by a reduction in seeking patterns in a drug-free no drug period. Notably, when animals have extended access to self-administration to the phase in which they can be identified as addicted-like (3-crit) and non-addicted-like (0-crit), a no

drug period at the start of the self-administration session is not sufficient to prevent maladaptive seeking expression (Deroche-Gamonet et al., 2004).

Adaptive inhibition over incited cocaine seeking

Despite the conserved ability of the ND DS to inhibit behavior in a drug free state, it was not sufficient to compete with the incentive motivational effects of non-contingent cocaine injections in a reinstatement protocol.

As previously shown (Belin et al., 2009), we confirm that cocaine-induced reinstatement under drug DS after early drug use is higher in male rats with a risk profile for addiction than in male rats with a resistant profile for addiction. We could not analyze it for females due to a limited cohort and number of high maladaptive seeking rats. When tested under ND DS, reinstatement was reduced but remained significant in vulnerable rats, while it was almost abolished in resistant rats.

While it is known that 3-crit rats show a higher cocaine-induced reinstatement than 0-crit rats under drug DS (Belin et al., 2009), it is still unknown whether 0-crit and 3-crit rats differ for cocaine-induced reinstatement under no drug DS, but it is most likely the case. In the transition to an addicted-like state, the no drug DS seems to lose its adaptive inhibitory value, including in a drug-free state. Whether this is a result of an increase in incentive control by the context or a progressive loss of control by inhibitory discriminatory stimuli is not known.

Conclusion

At this early stage, the external adaptive inhibitory DS can already not compete with neither the incentive motivational effects of a decrease in cocaine concentration (initiating a form of cocaine craving and seeking behavior during the no drug periods in the self-administration session) nor an increase in cocaine concentration (initiating a higher level of responding in cocaine-induced reinstatement).

At this early stage, the maladaptive behavior that is characteristic of vulnerable rats is driven by the incentive effect of cocaine and less than ideal intoxication levels. As cocaine administration has been shown to cause disruption of behavioral inhibition and conditional discrimination, it seems that the vulnerable rats may be more sensitive to cocaine's effects on disrupting conditional discrimination, leading to the consequential maladaptive behavior that is characteristic of a lack of proper behavioral inhibition (Paine & Olmstead, 2003).

As maladaptive seeking at this stage selectively emerges under cocaine effect, it seems that selectively in the rats vulnerable to developing addiction, the pharmacological influence of cocaine may have a stronger influence on discrimination, resulting in a reduced capacity to inhibit seeking behaviors. As pharmacokinetics has been shown to highly influence addiction liability and synaptic plasticity, the exploration of whether differences in maladaptive cocaine seeking as a consequence of poor discriminative ability is a result of pharmacokinetic influences has yet to be done.

CHAPTER 2: PHARMACOKINETIC INFLUENCES ON MALADAPTIVE COCAINE SEEKING

Drugs are drugs of abuse due to their pharmacological profile, with pharmacokinetics (PK) and pharmacodynamics (PD) having a strong influence on addiction liability (Farre & Cami, 1991).

Pharmacokinetics (PK) is defined as the movement of drugs through the body, whereas pharmacodynamics (PD) is defined as the body's biological response to drugs. PK describes a drug's absorption, distribution, metabolism, and excretion and PD describes how biological processes in the body respond to or are impacted by a drug. In other words, PK is what the body does to the drug and PD is what the drug does to the body.

PK describes a drug's exposure by characterizing its bioavailability as a function of time, PD describes a drug's response in terms of biochemical or molecular interactions. Logically PK and PD are not independent, and drug PK can influence drug PD and addiction liability- in the case of drugs of abuse. Particularly, the rate in which the drug reaches the brain (whether being due to different absorption rates of different drugs or different routes of administration for the same drug) influences its addictive potential, with the drugs reaching the brain the fastest being the most addictive. The rate (fast or slow injection speed) and pattern of cocaine intake (spontaneous or imposed low vs high frequency of intake, intermittent access compared to long access) also is evidenced to play a role in facilitating incentive motivation (Belin et al., 2009; Gueye et al., 2019; Kawa et al., 2016; Martín-García et al., 2014; Samaha et al., 2002). Changes in drug metabolism are also proven to successfully alter drug self-administration behavior (Zlebnik et al., 2014). Consequently, it has been evidenced that the PK factors such as the time to reach the peak drug concentration, the magnitude of the peak, and the frequency of peaks can influence addiction liability. However, questioning the contribution of how pharmacokinetic modulation (such as changes in distribution and metabolism) are associated with the development of addiction-like changes in incentive motivation and behavioral inhibition is rarely done.

First, only few studies have looked at the differences in distribution in the brain (Bystrowska et al., 2012), and to date, there have been no studies looking at differences of cocaine distribution in the brain as a function of addiction vulnerability. Most studies that analyze the importance of pharmacokinetics in cocaine addiction use a mathematical model of whole brain cocaine concentration (Pan et al., 1991; Zimmer et al., 2011). The use of this model does not encompass differences in cocaine concentration that has been previously observed in different structures. Importantly, the distribution can be affected by differences in transportation of cocaine across the blood brain barrier, which has been evidenced to be modulated by a novel cocaine-proton

antiporter (Chapy et al., 2015). Differences in distribution of cocaine in the brain could be an important and relatively unexplored factor influencing the drug-induced neuroadaptations.

Secondly, the influence of metabolism can be involved in either the promotion or protection from development of addiction-like changes. Briefly, cocaine metabolism results in production of three main metabolites (Xie et al., 1999). These metabolites are ecgonine methyl ester (EME), benzoylecgonine (BZE), and norcocaine. EME is a major metabolite found in urine and is produced by hydrolysis of cocaine in the blood by BChE. The second metabolite found in high amounts in urine is BZE, which is produced by spontaneous hydrolysis and by liver carboxylesterase (Brzezinski et al., 1994). Both EME and BZE are major metabolites, however they are inactive, meaning that they do not elicit pharmacological effects. Lastly, norcocaine is produced by demethylation by various cytochrome P450 (CYP) enzymes, however at much lower concentrations compared to EME or BZE (**Figure 18**). Norcocaine is a minor metabolite, however it is a highly hepatotoxic active metabolite that is able to cross the blood brain barrier (Roque Bravo et al., 2022).

It should be mentioned that there are significant differences in metabolism of cocaine between rats and humans. In rats, BChE, the enzyme responsible for production of EME from cocaine, is present in the plasma at 5% of the levels found in humans (Li et al., 2002), providing evidence for a potential limitation in translatability of this hypothesis.

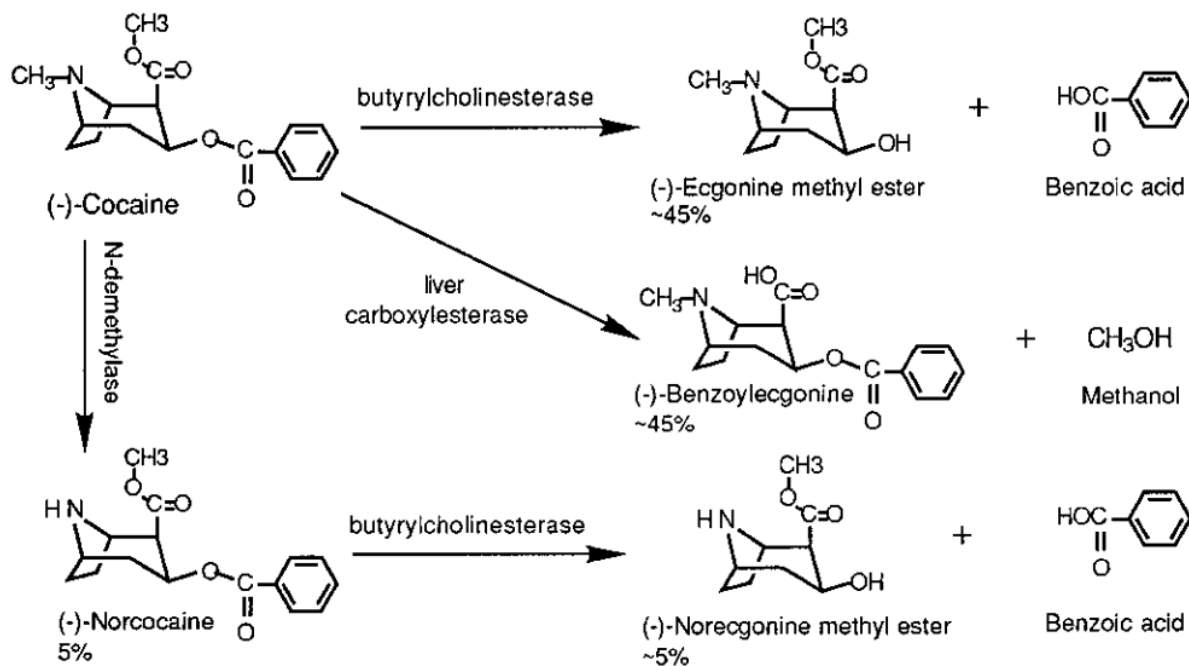


FIGURE 18: Metabolism of cocaine (from Xie et al., 1999)- Cocaine is broken down into three metabolites: ecgonine methyl ester (EME), benzoylecgonine (BZE), and norcocaine. EME is a major metabolite found in human urine and is produced by hydrolysis of cocaine in the blood by BChE. The second metabolite found in high amounts in human urine is BZE, which is produced by spontaneous hydrolysis and by liver carboxylesterase (Brzezinski et al., 1994). Lastly, norcocaine is produced by demethylation by various cytochrome P450 (CYP) enzymes, however at much lower concentrations compared to EME or BZE.

As shown in the previous chapter, modulation of internal state (cocaine concentration) highly influences maladaptive seeking expression early in the transition to addiction in a vulnerable subpopulation. As pharmacokinetic considerations influence addiction liability and cocaine use has been shown to negatively influence cognitive functioning, relationships between individual susceptibility of addiction and pharmacokinetic influences could highlight crucial mechanisms facilitating some of the behavioral handicaps of those vulnerable to developing addiction, including maladaptive seeking expression (Frazer et al., 2018).

In this chapter, individual variations in two facets of cocaine pharmacokinetics: distribution and metabolism are questioned.

MATERIALS AND METHODS

SUBJECTS

Male Sprague Dawley rats (experiment 1: n=21, experiment 2: n=20) weighing 250-300g at their arrival were single housed in a temperature ($22 \pm 1^\circ\text{C}$) and humidity ($60 \pm 5\%$) controlled vivarium on a reverse-light cycle (ON 19h00, OFF 7h00). Food and water were provided *ad libitum*. Rats were handled daily before experimentation began.

SPECIFIC DRUGS

Ketamine hydrochloride (80 mg/kg) (Imalgène 1000; Rhône Mérieux, Lyon, France) and xylazine hydrochloride (16 mg/kg) (Rompun; Rhône Mérieux, Lyon, France) were mixed with saline and administered intraperitoneally at a dose of 2 ml/kg of body weight. Exagon (200 mg/kg pentobarbital and 20 mg/kg lidocaine) was mixed with injectable water and administered intraperitoneally at a dose of 6.67 mL/kg of body weight. Cocaine hydrochloride was dissolved in sterile 0.9% NaCl.

SPECIFIC SURGERY

For the first experiment, two silastic catheters designated for either cocaine infusion or blood collection were implanted in the right and left jugular veins under ketamine anesthesia. The proximal end of the infusion catheter was passed under the skin and inserted into the right jugular vein, while the base emerged from the mid scapular region, while the proximal end of the collection catheter was inserted into the left jugular vein, passed under the skin, and the base emerged just posterior to the infusion catheter. Rats were allowed to recover for 5 to 9 days after surgery before self-administration began. For surgery conditions, see common methods. Experiment 1 aimed at evaluating brain and blood cocaine/metabolites, (the reason why two catheters were implanted was to prevent contamination of blood samples from self-administered cocaine). However, contrary to the results of brain quantification, the results from the blood quantification were highly unstable so they were not used.

For experiment 2, one catheter was implanted as described in the common methods.

SPECIFIC INTRAVENOUS SELF-ADMINISTRATION PROCEDURE

Basal self-administration sessions were run as described in the common methods.

Non-contingent infusion session for sacrifice

The session just before the animals were sacrificed consisted of a single drug period illuminated by the blue light, but with non-contingent infusions occurring at 5-minute intervals, starting at t=5 (**Figure 19**). The last infusion occurred as the session terminated. Active and inactive responses were recorded for the duration of the session but there were no scheduled consequences.

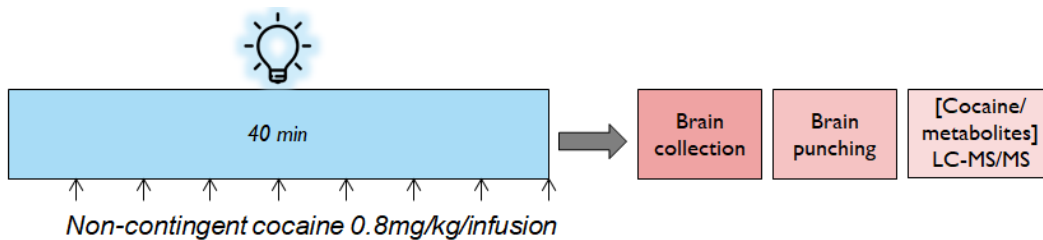


FIGURE 19: Sacrifice session for brain cocaine and metabolite quantification- On the day of sacrifice, all rats were placed in the self-administration chamber for forty minutes, receiving 8 non-contingent injections every five minutes. Immediately afterwards, they were sacrificed, and their brains were collected and snap frozen. Punching of the structures of interest was performed, then the cocaine and metabolite concentration was analyzed through LC-MS/MS.

BRAIN COLLECTION PROCEDURE

After a session with the non-contingent infusion protocol, animals were removed from the self-administration chambers, blood was collected, and then the rats were deeply anesthetized with Exagon. They were then perfused transcardially with 40 mL of sterile 0.9% physiological saline at a pump speed of 14 mL/minute. Brains were then removed, inserted into isopentane maintained at -50°C, and stored on dry ice. To avoid contamination, the isopentane solution was changed between animals.

BRAIN PUNCH PROCEDURE

Brains were thawed to -20°C and sliced on a cryostat (CM3050 S, Leica Microsystems, Wetzlar, Germany). The orbitofrontal cortex (OF), infralimbic cortex (IL), prelimbic cortex (PL), anterior cingulate cortex (ACC), dorsolateral striatum (DLS), dorsomedial striatum (DMS), nucleus

accumbens core (NacC), nucleus accumbens shell (NacSh), basolateral amygdala (BLA), habenula (HB), and ventral tegmental area (VTA) were punched using 1mm Integra Miltex biopsy punches (Integra, York, USA). A new biopsy punch was used for each structure and animal to avoid contamination. Left and right hemispheres were pooled, and the weight of the punches was recorded for subsequent cocaine concentration calculations.

COCAINE ANALYSIS PROCEDURE

Cocaine and its metabolites [norcocaine, benzoylecgonine (BZE) and ecgonine methyl ester (EME)] were analyzed through LC-MS/MS by Dr. Jean-Michel Gaulier and his team at CHRU Lille. Cocaine and related metabolites were assayed in brain samples (about 1 mg): each brain sample was incubated at 55 °C for 1 hour with 100 µL of a Subtilisine A (protease from bacillus licheniformis, Sigma, France) mixture at pH 7.4, and subsequently treated with ultrasound. Determination of cocaine and related metabolites in rat brain structures exhibited a lower limit of quantification of 0.025 ng/mg of brain tissue.

BLOOD COLLECTION PROCEDURE FOR PLASMA BUTYRYLCHOLINESTERASE ASSAY

Naïve

While rats were deeply anesthetized with ketamine/xylazine and being prepped for catheterization surgery, the tip of the tail was pierced using a clean 18G needle. Blood (0.2 mL) was collected in tubes coated with lithium heparin then centrifuged for 10 minutes at 2000 RPM at 4°C. Following centrifugation, plasma was collected and immediately put on dry ice. Samples were stored at -80°C until used for the BChE assay.

Sacrifice

While rats were deeply anesthetized with isoflurane, trunk blood was collected after decapitation. Blood (1 mL) was transferred to tubes coated with lithium heparin then centrifuged for 10 minutes at 2000 RPM at 4°C. Following centrifugation, plasma was collected and immediately put on dry ice. Samples were stored at -80°C until used for the BChE assay.

BUTYRYLCHOLINESTERASE ACTIVITY MEASUREMENT

BChE activity was measured with a DetectX® Butyrylcholinesterase Fluorescent Activity Kit (Arbor Assays, Ann Arbor, USA), which applies an end-point method. The reaction of BChE and its substrate, butyrylthiocholine iodide, produces choline and butyrate. A non-fluorescent molecule, Thiostar®, converts to a brightly fluorescent product upon binding and reacting with the thiol groups that were made available by the BChE/substrate reaction. Trials were made and optimal conditions were defined as following: Plasma samples were diluted 50-fold and read in duplicate with an excitation wavelength of 355 nm and an emission wavelength of 520 nm at 25°C using a POLARstar® Omega spectrophotometer (BMG Labtech, ref. 415-1479).

EXPERIMENTAL TIMELINE

Experiment 1: Brain cocaine concentration

In experiment 1 (n=21), the rats self-administered cocaine for either 26 or 27 basal self-administration sessions before the sacrifice session. As mentioned in the methods, on the day of sacrifice, the rats were placed in the self-administration chamber and were administered 8 unsignaled non-contingent injections at the self-administered dose (0.8 mg/kg/46µL) over 40 minutes (1 infusion every 5 minutes)

Experiment 2: Plasma butyrylcholinesterase activity

In experiment 2 (n=20), blood from the tail was collected during surgery. After, the rats self-administered cocaine for 25 basal self-administration sessions before being sacrificed 24 hours in withdrawal. During sacrifice (under deep anesthesia and after decapitation), blood was collected from the trunk and brains were used for a molecular biology project.

SPECIFIC DATA AND STATISTICAL ANALYSIS

Self-administration behavior

Variables of interest were: Inter-infusion interval, injections, and active nose-pokes during the first no drug period, mean active no drug responding for sessions 1-6, mean active no drug responding for sessions 7-17.

Cocaine and metabolite distribution analysis

Variables of interest were: Concentration of cocaine, EME, BZE, and norcocaine

Cocaine and metabolite distribution was analyzed using repeated measures ANOVA with structure (IL, PL, OF, ACC, DLS, DMS, NacC, NacSh, BLA, HB, VTA) as within-subject factors. Significant main effects or interactions were explored by pairwise comparisons of means using the Fisher LSD post-hoc test.

BChE

Variables of interest were: BChE activity (naïve), BChE activity (sacrifice). Comparison of BChE activity as a function of time was done by a paired t test.

Criteria for identification of maladaptive seeking rats

See common methods

Criteria for identification of subpopulations- high/low [cocaine]

Variables of interest were: Concentration of cocaine in the PL.

High and low [cocaine] groups were determined by taking the 8 individuals with the highest and lowest cocaine concentration.

Group comparisons

Rats identified as High and Low seeking rats were compared as described in the common methods.

Comparison of variables between groups (high [cocaine] vs low [cocaine]) during the self-administration session the day before sacrifice was performed by unpaired t tests.

Comparison of the evolution of no drug responding was done using a repeated measures ANOVA with time (sessions 1-6 vs sessions 7-17) as within-subject factors and groups (high [cocaine] vs low [cocaine]) as between-subject factors.

RESULTS

BRAIN COCAINE AND METABOLITES: RELATIONSHIP WITH SELF-ADMINISTRATION BEHAVIOR

Acquisition of self-administration behavior

Rats acquired cocaine self-administration as shown by both the significant discrimination between the active and inactive holes [ANOVA, hole effect: $F(1,19)=69.204$, $p<0.0001$] increasing over sessions [hole x session effect: $F(16, 304)=8.3663$, $p<0.0001$] and an increase in the number of cocaine injections per session which achieved stability in the population by day 10 [N=21, ANOVA, session effect: $F(16, 304)=18.216$, $p<0.0001$] [Fisher LSD post-hoc].

Regarding no drug periods, we analyzed here the first one (as this is the one that will be used for rats' classification as high and low maladaptive seekers). In this period, a higher number of responding in the active hole was also observed [ANOVA, hole effect: $F(1, 17)=32.599$, $p<0.0001$] with fluctuation over the sessions [hole x session effect: $F(16, 272)=3.224$, $p<0.0001$].

Identification and classification of maladaptive seeking groups

Classification of high and low seekers was done on the average no drug responses on days 15-17.

As previously mentioned, in acquisition, responding and discrimination evolved over time differently between the drug and no drug periods. In the drug period, this relationship did not differ between high and low seekers [Drug: hole x session x maladaptive group effect: $F(16, 304)=1.5373$, $p=0.08543$] but it did in the no drug period [No drug 1: hole x session x maladaptive group effect: $F(16, 272)=1.8060$, $p<0.05$] indicative of a progressive development of maladaptive seeking in high seeker rats starting at session 7 (data not shown).

Injections over the acquisition period differed between maladaptive seeking groups [ANOVA, maladaptive group effect: $F(1, 19)=5.1436$, $p<0.05$] with high seekers showing a higher number of injections but the trajectory did not differ over sessions [ANOVA, session x maladaptive group effect: $F(16, 304)=0.96507$, $p=0.49517$].

As expected, over the sessions used to characterize them, the high (n=10) and low (n=11) groups differed for the average total active no drug nose-pokes ($p<0.0001$)

(**Figure 20A**). In accordance with the observed effect during the whole acquisition phase, high no drug seekers also showed a higher number of average total injections (**Figure 20B**) and a lower average inter-infusion interval over these sessions (**Figure 20C**) (injections $p < 0.05$, III $p < 0.05$).

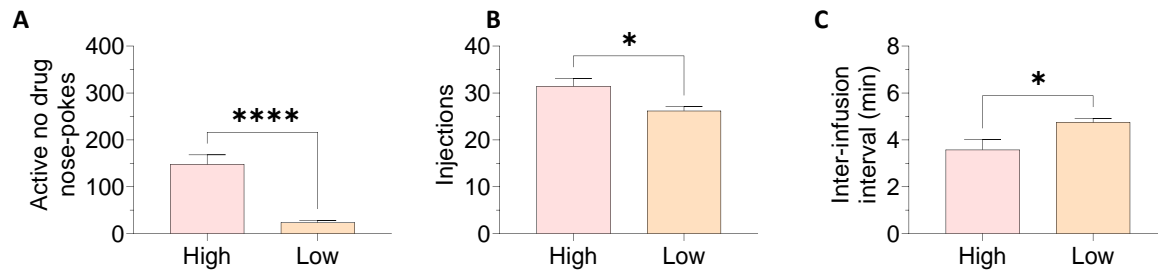


FIGURE 20: Brain cocaine/metabolite experiment: self-administration behavior in high and low maladaptive seekers- High and low seekers differed in A. total active nose-pokes, B. total injections, and C. inter-infusion interval during the sessions used to characterize them as high or low. **** $p < 0.0001$, * $p < 0.05$

Brain cocaine and metabolites concentration

After identical non-contingent cocaine infusions in all animals, significant variation was observed in cocaine and metabolite concentration between the collected brain regions (structure effect, $p < 0.0001$) (**Figure 21A-D**). On average, the highest concentration of cocaine was found in the PL, however it was also the structure with the highest variation. The PL also contained the highest concentration of all metabolites. The structure with the lowest cocaine and metabolite concentration was the VTA. Variations between individuals were also observed, which were consistent across structures as shown by high positive correlations between structures for both cocaine and metabolites.

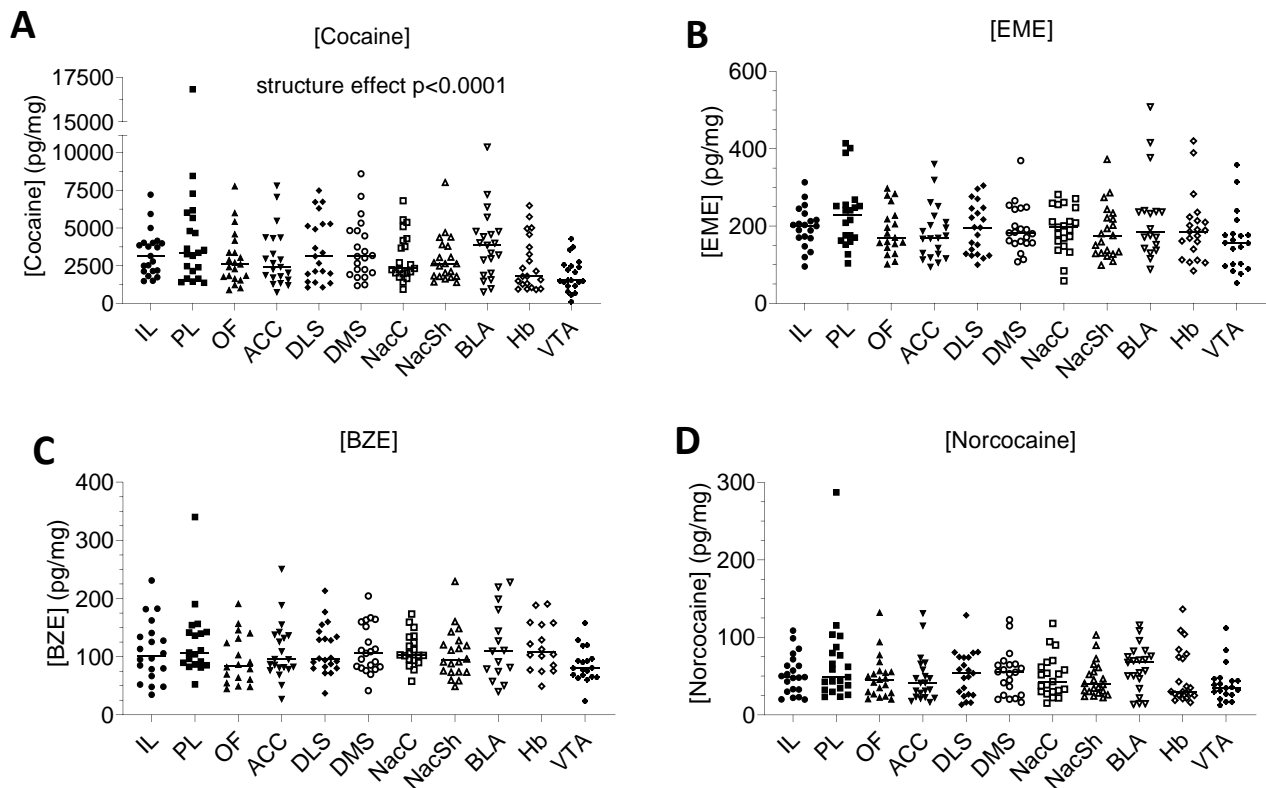


FIGURE 21: Cocaine and metabolite concentration in the brain regions collected after non contingent cocaine infusions- All rats were administered with 8 non contingent cocaine iv infusions, one every 5 min, over 40 min, and sacrificed immediately after for brain collection. A. Cocaine, B. EME, C. BZE, and D. Norcocaine concentration found in each brain region.

Relationship between cocaine and metabolites

Generally, the more cocaine in the brain was associated with a higher level of metabolites. This was concluded by performing correlations between the concentrations of cocaine in each brain region with the metabolite concentrations (**Table 2**). Brain cocaine distribution was correlated with all metabolites in the IL, PL, OF, DLS, DMS, NacC, BLA, Hb, and VTA. In the ACC and NacSh, cocaine was not correlated with BZE (ACC: $r=0.3352$, $r^2=0.1124$, $p=0.137$; NacSh: $r=0.2750$, $r^2=0.0456$, $p=0.2407$) but was correlated with both EME and norcocaine. The explanation for this lack of relationship between cocaine and BZE is not known, but it can possibly be due to individual differences in the metabolic activity in different structures, and in this case, less

carboxylesterase activity in these structures. This makes sense, as the carboxylesterase activity is mainly in the liver and not the brain, whereas BChE (the enzyme responsible for producing EME) and N-demethylases (the family of enzymes responsible for producing norcocaine) can cross the blood brain barrier more easily (Roque Bravo et al., 2022). The relationship between cocaine and norcocaine is much closer than the other metabolites, but this is likely due to the widespread presence of drug metabolizing enzymes with N-demethylase activity (cytochrome P450) in the brain.

	EME	BZE	Norcocaine
IL [cocaine]	R=0.5768 r ² =0.3327 p=0.008 N=20	R=0.5559 r ² =0.3090 p=0.011 N=20	R=0.7999 r ² =0.6399 p=0.000 N=21
PL [cocaine]	R=0.7189 r ² =0.5168 p=0.000 N=20	R=0.8687 r ² =0.7546 p=0.000 N=19	R=0.9496 r ² =0.9017 p=0.000 N=21
OF [cocaine]	R=0.5613 r ² =0.3151 p=0.012 N=19	R=0.5597 r ² =0.3132 p=0.016 N=18	R=0.8733 r ² =0.7627 p=0.000 N=21
ACC [cocaine]	R=0.5610 r ² =0.3148 p=0.008 N=21	R=0.3352 r ² =0.1124 p=0.137 N=21	R=0.9245 r ² =0.8548 p=0.000 N=21
DLS [cocaine]	R=0.5679 r ² =0.3225 p=0.007 N=21	R=0.5777 r ² =0.3337 p=0.006 N=21	R=0.8654 r ² =0.7489 p=0.000 N=21
DMS [cocaine]	R=0.6376 r ² =0.4065 p=0.002 N=21	R=0.4894 r ² =0.2396 p=0.024 N=21	R=0.8816 r ² =0.7772 p=0.000 N=21
NacC [cocaine]	R=0.4661 r ² =0.2172 p=0.033 N=21	R=0.6857 r ² =0.4702 p=0.001 N=19	R=0.8385 r ² =0.7031 p=0.000 N=21
NacSh [cocaine]	R=0.5429 r ² =0.2947 p=0.011 N=21	R=0.2750 r ² =0.0456 p=0.2407 N=20	R=0.8372 r ² =0.7010 p=0.000 N=21
BLA [cocaine]	R=0.7420 r ² =0.5505 p=0.000 N=18	R=0.7553 r ² =0.5704 p=0.000 N=15	R=0.8474 r ² =0.7181 p=0.000 N=21
HB [cocaine]	R=0.7324 r ² =0.5364 p=0.000 N=21	R=0.7027 r ² =0.4938 p=0.002 N=16	R=0.9384 r ² =0.8806 p=0.000 N=21
VTA [cocaine]	R=0.7745 r ² =0.5998 p=0.000 N=20	R=0.6578 r ² =0.4327 p=0.002 N=19	R=0.8727 r ² =0.7615 p=0.000 N=20

TABLE 2 : Correlations of [cocaine] and [metabolites] in each brain region after non contingent cocaine infusions- All rats were administered with 8 non contingent cocaine iv infusions, one every 5 min, over 40 min, and sacrificed immediately after for brain collection. Table containing correlations between the cocaine concentration and EME, BZE, and norcocaine levels. All structures had correlations between [cocaine] and [EME] / [cocaine] and [norcocaine]. All structures except ACC and NacSh had correlations between [cocaine] and [BZE].

Brain cocaine concentration and body weight

Brain cocaine concentration was not related to body weight in most brain regions (**Table 3**). In the VTA, there was an inverse correlation between the body weight of the animal at sacrifice and the concentration of cocaine ($r=-0.5307$, $r^2=0.2816$, $p<0.05$) (**Figure 22**). However, in other brain regions, this relationship did not exist.

	Body weight
IL [cocaine]	R=-0.1747 $r^2=0.0305$ $p=0.449$ N=21
PL [cocaine]	R=-0.2177 $r^2=0.0474$ $p=0.343$ N=21
OF [cocaine]	R=-0.3571 $r^2=0.1275$ $p=0.112$ N=21
ACC [cocaine]	R=-0.2955 $r^2=0.0873$ $p=0.193$ N=21
DLS [cocaine]	R=-0.0854 $r^2=0.0073$ $p=0.713$ N=21
DMS [cocaine]	R=-0.1657 $r^2=0.0275$ $p=0.473$ N=21
NacC [cocaine]	R=0.0597 $r^2=0.0036$ $p=0.797$ N=21
NacSh [cocaine]	R=0.1440 $r^2=0.0207$ $p=0.534$ N=21
BLA [cocaine]	R=0.2087 $r^2=0.0436$ $p=0.364$ N=21
HB [cocaine]	R=-0.1640 $r^2=0.0269$ $p=0.478$ N=21
VTA [cocaine]	R=-0.5307 $r^2=0.2816$ $p=0.013$ N=21

TABLE 3: Correlations between [cocaine] and body weight- Cocaine concentration was not correlated to body weight in any structure apart from the VTA.

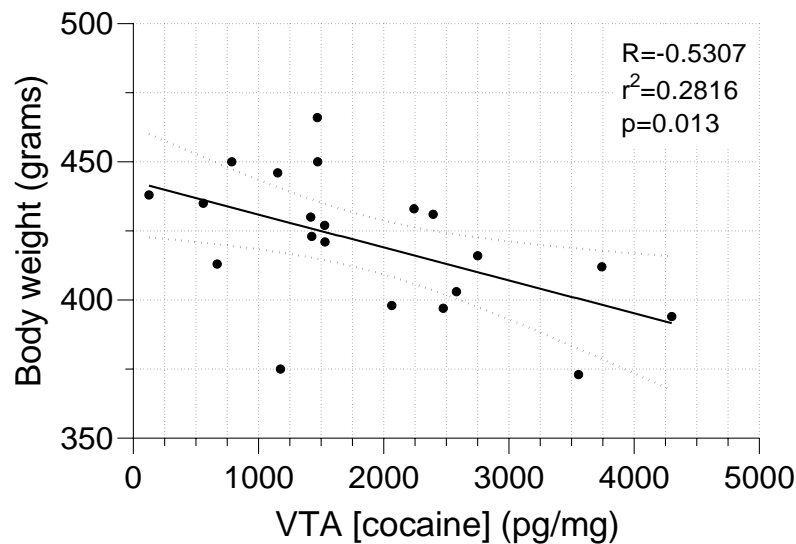


FIGURE 22: Relationship between VTA [cocaine] and body weight- There was a correlation between the concentration of cocaine in the VTA and the body weight of the animal at sacrifice.

Brain cocaine distribution: relationship with intake behavior in self-administration

To establish relationships between intake behavior in self-administration and brain cocaine concentration after equal non contingent cocaine administration, we chose the last basal self-administration session preceding sacrifice as it was the most recent self-administration session where the animals could behave freely and receive contingent cocaine injections.

In most of the brain structures studied, cocaine concentration after identical non-contingent cocaine infusions was negatively related to total cocaine intake (total number of injections) and positively related to the rate of intake (measured by the inter-infusion interval or III) during the session preceding sacrifice. The higher the cocaine concentration, the lower the cocaine intake and the slower the pattern of intake (long III). Such correlations were observed for the IL, PL, DLS, DMS, NacC, and HB. Only tendencies or significance for only one variable were observed for the OF, NacSh, ACC and BLA. Solely the cocaine concentration in the VTA was not correlated with cocaine intake and pattern of self-administration (**Table 4**).

In summary and globally, cocaine distribution appears to impact how fast and how much a rat self-administers cocaine.

	III total	Injections total
IL [cocaine]	R=0.5833 r ² =0.3402 p=0.006 N=21	R=-0.5915 r ² =0.3499 p=0.005 N=21
PL [cocaine]	R=0.5906 r ² =0.3512 p=0.005 N=21	R=-0.5440 r ² =0.2959 p=0.011 N=21
OF [cocaine]	R=0.4274 r ² =0.1827 p=0.053 N=21	R=-0.3920 r ² =0.1537 p=0.079 N=21
ACC [cocaine]	R=0.3981 r ² =0.1585 p=0.074 N=21	R=-0.3617 r ² =0.1308 p=0.107 N=21
DLS [cocaine]	R=0.4523 r ² =0.2046 p=0.040 N=21	R=-0.4837 r ² =0.2340 p=0.026 N=21
DMS [cocaine]	R=0.4488 r ² =0.2014 p=0.041 N=21	R=-0.4543 r ² =0.2064 p=0.039 N=21
NacC [cocaine]	R=0.4730 r ² =0.2237 p=0.030 N=21	R=-0.4797 r ² =0.2301 p=0.028 N=21
NacSh [cocaine]	R=0.3974 r ² =0.1579 p=0.075 N=21	R=-0.3995 r ² =0.1596 p=0.073 N=21
BLA [cocaine]	R=0.4131 r ² =0.1707 p=0.063 N=21	R=-0.4747 r ² =0.2253 p=0.030 N=21
HB [cocaine]	R=0.5150 r ² =0.2652 p=0.017 N=21	R=-0.5774 r ² =0.3334 p=0.006 N=21
VTA [cocaine]	R=0.2861 r ² =0.0818 p=0.209 N=21	R=-0.2450 r ² =0.0600 p=0.285 N=21

TABLE 4: Correlations between [brain cocaine] and cocaine intake- All rats were administered with 8 non contingent cocaine iv infusions, one every 5 min, over 40 min, and sacrificed immediately after for brain collection. Brain cocaine concentration were related to self-administration behavior previously expressed. Correlations were observed in the IL, PL, DLS, DMS, NacC, and HB between [cocaine] and both inter-infusion interval and total injections over the whole self-administration session before sacrifice. In the BLA, there was a correlation between [cocaine] and total injections, however the correlation was not significant for III. In the OF, ACC, NacSh, and VTA, [cocaine] was not correlated with neither inter-infusion interval nor total injections.

Brain cocaine distribution: relationship with maladaptive seeking behavior in self-administration

There was no correlational relationship between the cocaine concentration in any of the brain regions analyzed and the pattern of no drug responding during the final basal self-administration session before sacrifice, indicating that brain cocaine distribution was not quantitatively related to adaptive/maladaptive seeking.

Prelimbic cocaine distribution: relationship with intake and maladaptive seeking behavior in self-administration

As the PL was found to have the highest concentration of cocaine and metabolites and it is hypothesized to have crucial involvement in maladaptive cocaine seeking in vulnerable animals, exploration of the distribution of cocaine in the PL was explored further. When the rats were split in groups determined by the amount of cocaine found in the PL, in accordance with the correlation data, there was a significant difference between the inter-infusion interval (**Figure 23A**) and total number of injections (**Figure 23C**) between the subpopulations (III $p < 0.005$, injections $p < 0.01$), with the rats with the lowest cocaine concentration showing a fast pattern of intake (short III) (**Figure 23B**) and a higher number of cocaine injections (**Figure 23D**).

However, when these two subpopulations were compared on the average active nose-pokes in the first no drug period during the session the day before sacrifice, there was no significance ($p = 0.1778$) (**Figure 23E**). Supporting this, there was no correlation between cocaine concentration in the PL and the average active nose-pokes in the first no drug period in the session before sacrifice (**Figure 23F**).

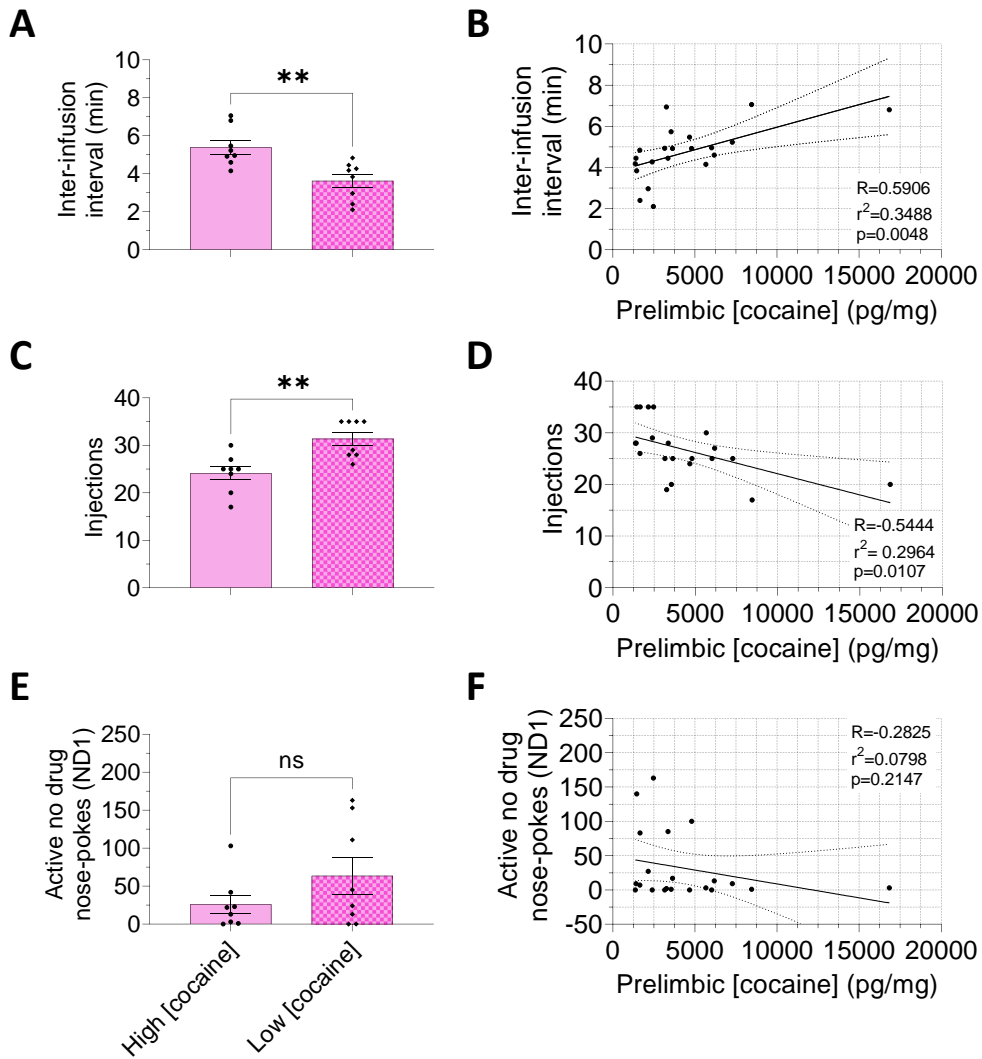


FIGURE 23: Relationship between PL cocaine concentration and self-administration behaviors- A. Rats with high cocaine concentration found in the PL after equal non-contingent cocaine had slower rates of intake in the self-administration session the day before sacrifice than those with low cocaine concentration. B. There was a positive correlation between the inter-infusion interval in the session the day before sacrifice and the concentration of cocaine in the PL. C. Rats with high cocaine concentration found in the PL had significantly higher intake in the session the day before sacrifice than those with low cocaine concentration. D. There was a negative correlation between the number of cocaine injections in the session the day before sacrifice and the concentration of cocaine in the PL. E. Rats with high and low cocaine concentration found in the PL had no difference in active nose-pokes during the first no drug period in the session the day before sacrifice. F. Unlike inter-infusion interval and injections, there was no relationship between PL cocaine concentration and active nose-pokes in the first no drug period in the session the day before sacrifice between rats with high and low cocaine in the PL.

A deeper look into the maladaptive seeking patterns of the high and low brain cocaine animals uncovered a trend in the evolution of no drug responding. It was found that rats having low PL cocaine had a nearly significant change in active hole no drug responses between the first 6 sessions and the subsequent ten sessions ($p=0.0516$) while this evolution was not observed in rats that had high cocaine found in the PL (**Figure 24**). In summary, variations in brain cocaine distribution appeared to be potentially related to a decrease over time in adaptive inhibitory control over seeking during ND periods.

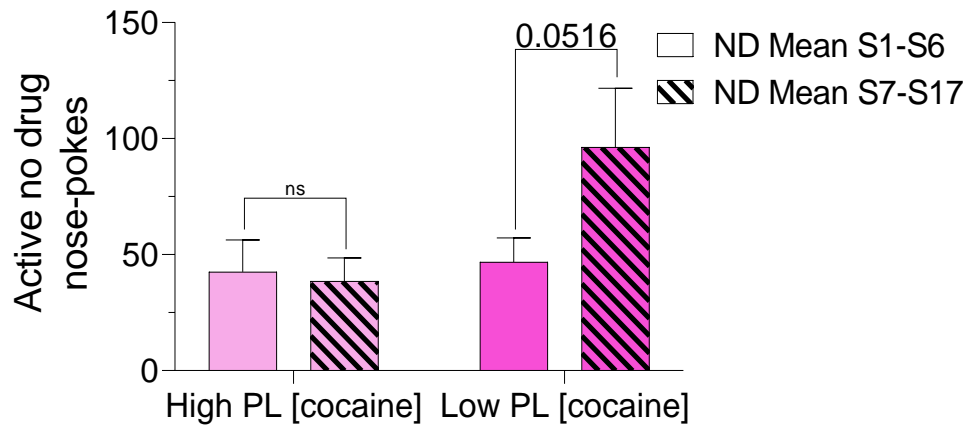


FIGURE 24: Evolution of no drug responding in rats with high and low cocaine in the PL- A. Rats with high PL cocaine did not have an evolution of responding in the no drug period between the first 6 sessions and the subsequent 10 sessions, whereas the rats with low PL cocaine had a nearly significant ($p=0.0516$) difference in the active nose-pokes between the two time points.

Maladaptive cocaine seeking: relationship with PL cocaine distribution

Starting from behavior, instead of cocaine concentration, we explored whether rats identified as high and low maladaptive seekers differed for cocaine concentration in the PL. No significant difference was observed despite a trend ($p=0.1879$) (**Figure 25**).

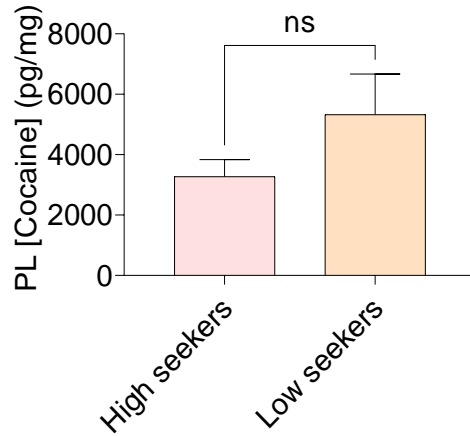


FIGURE 25: Comparison of brain PL cocaine concentration as a function of maladaptive seeking group- High and low maladaptive seekers did not differ in PL cocaine concentration ($p=0.1879$).

Brain metabolite/cocaine ratios and intake behavior in self-administration

The ratio of cocaine to metabolites was analyzed to determine if there was variation in the different pathways in which cocaine can be degraded. This was done by creating a ratio of each metabolite (EME, BZE, and norcocaine) to cocaine (Lane & Levy, 1981). These ratios were then related to self-administration behavior to try and identify differences in cocaine breakdown as a factor of intake patterns and maladaptive seeking expression. While not universally significant, the ratio between EME and cocaine and self-administration behavior reached significance the most frequently (**Table 5**). The ratio between BZE and cocaine reached significance for some structures and intake behaviors (**Table 6**). The relationship between the ratio of norcocaine to cocaine and intake patterns seems separate, as not a single behavior or region was correlated (**Table 7**).

[EME]/[cocaine]			
Structure	III total	Injections total	ND1 active nose-pokes
IL	R ² =0.365440368, r=-0.60451664, p=0.00475263 N=20	R ² =0.496845, r=0.704872, p=0.000519 N=20	R ² =0.392078, r=0.626161, p=0.003141 N=20
PL	R ² =0.224841, r=-0.474174, p=0.034666 N=20	R ² =0.307748241, r=0.554750612, p=0.011127534 N=20	R ² =0.135368, r=0.367924, p=0.110482 N=20
OF	R ² =0.170203, r=-0.412557, p=0.079196 N=19	R ² =0.231297, r=0.480934, p=0.037113 N=19	R ² =0.149040, r=0.386057, p=0.102562 N=19
ACC	R ² =0.108754, r=-0.329779, p=0.144317 N=21	R ² =0.149391, r=0.386511, p=0.083495 N=21	R ² =0.005046, r=0.071036, p=0.759619 N=21
DLS	R ² =0.014641, r=-0.120998, p=0.601353 N=21	R ² =0.049725, r=0.222992, p=0.331243 N=21	R ² =0.003277, r=0.057243, p=0.805328 N=21
DMS	R ² =0.120250, r=-0.346771, p=0.123550 N=21	R ² =0.174148, r=0.417311, p=0.059811 N=21	R ² =0.038063, r=-0.195097, p=0.396723 N=21
NacC	R ² =0.170286, r=-0.412658, p=0.063015 N=21	R ² =0.210619, r=0.458932, p=0.036378 N=21	R ² =0.037636, r=0.194000, p=0.399434 N=21
NacSh	R ² =0.256696, r=-0.506652, p=0.019085 N=21	R ² =0.261970, r=0.511830, p=0.017699 N=21	R ² =0.080817, r=0.284284, p=0.211685 N=21
BLA	R ² =0.188952, r=-0.434686, p=0.071439 N=18	R ² =0.289561, r=0.538109, p=0.021245 N=18	R ² =0.238836, r=0.488708, p=0.039591 N=18
HB	R ² =0.099442, r=-0.315344, p=0.163792 N=21	R ² =0.184587, r=0.429636, p=0.051924 N=21	R ² =0.093666, r=0.306048, p=0.177256 N=21
VTA	R ² =0.135196, r=-0.367690, p=0.110726 N=20	R ² =0.178033, r=0.421939, p=0.063869 N=20	R ² =0.095606, r=0.309202, p=0.184660 N=20

TABLE 5 : Correlation of [EME]/[cocaine] after non contingent cocaine infusions and self-administration behavior- Table containing correlations between the [EME]/[cocaine] ratio and average inter-infusion interval, total injections, and no drug active nose-pokes (ND1) in the session before sacrifice.

[BZE]/[cocaine]			
Structure	III total	Injections total	ND1 active nose-pokes
IL	R ² =0.133599318, r= -0.365512405, p=0.113017297 N=20	R ² =0.065887, r=0.256684, p=0.274637 N=20	R ² =0.027566, r=0.166029, p=0.484194 N=20
PL	R ² =0.027542, r=-0.165958, p=0.484383 N=20	R ² =0.011390, r=0.106725, p=0.654270 N=20	R ² =0.001436, r=0.037899, p=0.873958 N=20
OF	R ² =0.086831, r=-0.294670, p=0.235223 N=18	R ² =0.167378, r=0.409119, p=0.091824 N=18	R ² =0.376089, r=0.613261, p= 0.006800 N=18
ACC	R ² =0.0902463, r= -0.300410298, p=0.185780395 N=21	R ² = 0.166260384, r= 0.407750394, p= 0.0665336371 N=21	R ² =0.188737, r=0.434439, p= 0.049076 N=21
DLS	R ² = 0.031697, r= -0.178036, p= 0.440051 N=21	R ² =0.0614949381, r= 0.247981729, p=0.278430302 N=21	R ² =0.067217, r=0.259262, p=0.256426 N=21
DMS	R ² = 0.093254327, r=-0.3053757, p=0.17825837 N=21	R ² =0.129772174, r= 0.360239051, p=0.10868454 N=21	R ² =0.013864, r=0.117745, p=0.611237 N=21
NacC	R ² =0.108499, r=-0.329391, p=0.168480 N=19	R ² =0.131615, r=0.362787, p=0.126872 N=19	R ² =0.251022, r=0.501021, p= 0.028877 N=19
NacSh	R ² =0.227564, r=-0.477037, p= 0.033438 N=20	R ² =0.300559, r=0.548233, p= 0.012324 N=20	R ² =0.252526, r=0.502520, p= 0.023939 N=20
BLA	R ² =0.275334, r=-0.524723, p= 0.044617 N=15	R ² =0.261031, r=0.510912, p=0.051620 N=15	R ² =0.154826, r=0.393479, p=0.146770 N=15
HB	R ² =0.379348, r=-0.615912, p= 0.011076 N=16	R ² =0.402530, r=0.634452, p= 0.008294 N=16	R ² =0.237256, r=0.487089, p=0.055674 N=16
VTA	R ² =0.121280, r=-0.348252, p=0.143977 N=19	R ² =0.222756, r=0.471971, p= 0.041327 N=19	R ² =0.143824, r=0.379242, p=0.109302 N=19

TABLE 6 : Correlation of [BZE]/[cocaine] after non contingent cocaine infusions and self-administration behavior- Table containing correlations between the [BZE]/[cocaine] ratio and average inter-infusion interval, total injections, and no drug active nose-pokes (ND1) in the session before sacrifice.

[norcocaine]/[cocaine]			
Structure	III total	Injections total	ND1 active nose-pokes
IL	R ² = 0.00618, r=-0.078641, p=0.7347 N=21	R ² = 0.015937, r=-0.126244, p=0.585556 N=21	R ² =0.006691, r=-0.081797, p=0.724480 N=21
PL	R ² =0.000062, r=-0.007887, p=0.972933 N=21	R ² =0.000007, r=-0.002597, p=0.991087 N=21	R ² =0.002183, r=0.046725, p=0.840605 N=21
OF	R ² =0.014628, r=-0.120948, p=0.601505 N=21	R ² =0.014274, r=-0.119472, p=0.605981 N=21	R ² =0.025118, r=-0.158486, p=0.492613 N=21
ACC	R ² =0.007841, r=0.088547, p=0.702700 N=21	R ² =0.018280, r=-0.135205, p=0.558989 N=21	R ² =0.001882, r=0.043380, p=0.851891 N=21
DLS	R ² =0.001844, r=0.0429, p=0.853 N=21	R ² =0.009990, r=-0.099952, p=0.666414 N=21	R ² =0.003158, r=-0.056195, p=0.808827 N=21
DMS	R ² =0.0368, r=0.1919, p=0.4046 N=21	R ² =0.052947, r=-0.230102, p=0.315648 N=21	R ² =0.004066, r=-0.063763, p=0.783633 N=21
NacC	R ² =0.0760, r=0.2757, p=0.2264 N=21	R ² =0.065407, r=-0.255749, p=0.263157 N=21	R ² =0.034810, r=-0.186575, p=0.418063 N=21
NacSh	R ² =0.004596, r=0.0678, p=0.7703 N=21	R ² =0.007617, r=-0.087276, p=0.706785 N=21	R ² =0.001762, r=0.041978, p=0.856628 N=21
BLA	R ² =0.01934, r=0.1391, p=0.5477 N=21	R ² = 0.032148, r=-0.179300, p=0.436759 N=21	R ² =, 0.027722 r=-0.166498, p=0.470707 N=21
HB	R ² =0.001142, r=-0.03379, p=0.8844 N=21	R ² =0.000000, r=-0.000261, p=0.999103 N=21	R ² =0.004954, r=0.070388, p=0.76175 N=21
VTA	R ² =0.002819, r=0.053093, p=0.824077 N=20	R ² =0.000565, r=-0.023776, p=0.920743 N=20	R ² =0.003362, r=-0.057983, p=0.808148 N=20

TABLE 7 : Correlation of [norcocaine]/[cocaine] after non contingent cocaine infusions and self-administration behavior- Table containing correlations between the [norcocaine]/[cocaine] ratio and average inter-infusion interval, total injections, and no drug active nose-pokes (ND1) in the session before sacrifice.

PLASMA BUTYRYLCHOLINESTERASE ACTIVITY: RELATIONSHIP WITH SELF-ADMINISTRATION BEHAVIOR

Acquisition of self-administration behavior

Rats acquired cocaine self-administration as shown by both the significant discrimination between the active and inactive holes [ANOVA, hole effect: $F(1, 18)=25.192$, $p<0.0001$] increasing over sessions [hole x session effect: $F(16, 288)=4.9740$, $p<0.0001$] and an increase in the number of cocaine injections per session which achieved stability in the population by day 9 [N=20, ANOVA, session effect: $F(16, 272)=8.5101$, $p<0.0001$] [Fisher LSD post-hoc].

Regarding no drug periods, we analyzed here the first one (as this is the one that will be used for rats' classification as high and low maladaptive seekers). In this period, a higher number of responding in the active hole was also observed [ANOVA, hole effect: $F(1, 12)=18.384$, $p<0.005$] with fluctuation over the sessions [hole x session effect: $F(16, 192)=2.1098$, $p<0.01$].

Identification and classification of maladaptive seeking groups

Classification of high and low seekers was done on the average no drug responses on days 15-17.

As previously mentioned, in acquisition, responding and discrimination evolved over time differently between the drug and no drug periods. However, in both the drug and no drug period, this relationship did not differ between high and low seekers [Drug: hole x session x maladaptive group effect: $F(16, 288)=0.70$, $p=0.78$, No drug 1: hole x session x maladaptive group effect: $F(16, 192)=0.44$, $p=0.97$].

Neither mean injections per session [ANOVA, maladaptive group effect: $F(1, 18)=0.03$, $p=0.85$] nor the trajectory of injections over the acquisition period differed as a function of group [ANOVA, session x maladaptive group effect: $F(16, 288)=0.70$, $p=0.79$].

As expected, the high (n=12) and low (n=8) groups differed for the average total active no drug nose-pokes ($p<0.005$) over the sessions used to characterize them as high and low seekers. On these same sessions, they did not differ for the number of average total injections nor for the average inter-infusion interval (injections $p=0.68$, III $p=0.90$).

Butyrylcholinesterase activity and body weight

Blood was collected for the BChE assay prior to (naïve condition) and after experience with cocaine self-administration (sacrifice condition). BChE activity was not found to be related to the body weight of the animal in the naïve condition (**Figure 26A**) nor the sacrifice condition (**Figure 26B**) (Naïve: $r=0.4229$, $r^2=0.1788$, $p=0.063$, Sacrifice: $r=0.0969$, $r^2=0.0094$, $p=0.684$). However, in the naïve condition, there seemed to be a stronger relationship between the body weight of the rat and BChE activity.

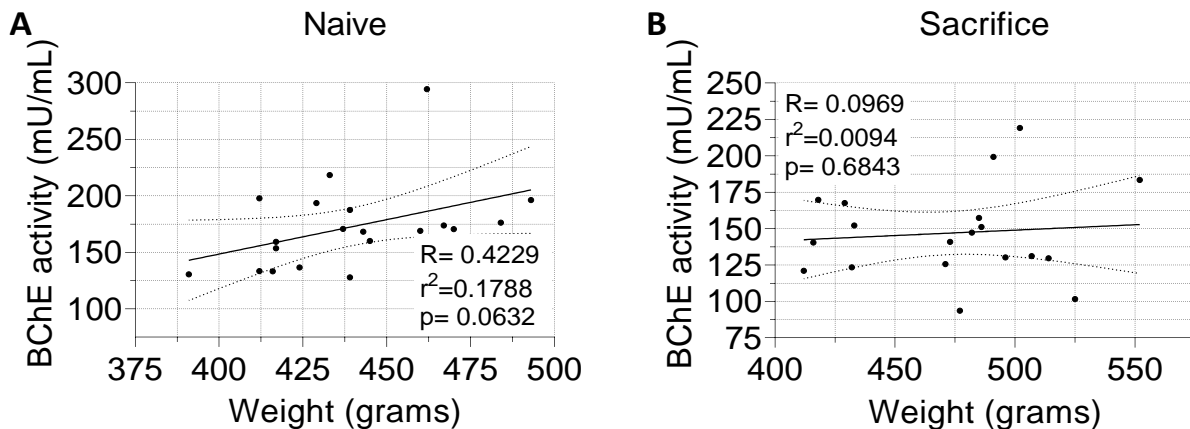


FIGURE 26: Relationship of BChE activity and body weight- A. There was a tendency in the naïve condition that the activity of BChE was related to the body weight of the animal. B. At sacrifice, this relationship was not maintained.

Effect of cocaine exposure on butyrylcholinesterase levels in the plasma

Cocaine exposure led to a significant decrease in BChE activity. ($p<0.01$) (**Figure 27**).

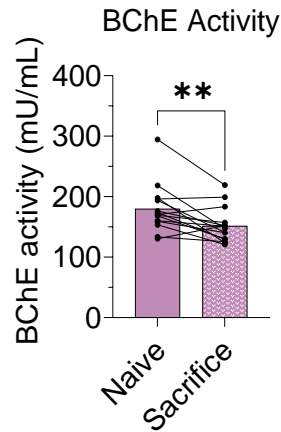


FIGURE 27: BChE activity before and after cocaine self-administration- A. There was a significant decrease in BChE activity after 25 days of intravenous cocaine self-administration. ** $p < 0.01$

Butyrylcholinesterase activity and cocaine intake pattern

BChE activity from naïve samples were not found to be related to rate of intake or other vulnerability markers from their self-administration behavior. In naïve samples, there was no relationship between the initial rate of intake (average inter-infusion interval on sessions 2 and 3) and plasma BChE levels (**Figure 28A**) ($N=20$, $r=0.1661$, $r^2=0.0276$, $p=0.4839$).

However, when the plasma BChE was measured in samples at the end of the experiment after cocaine self-administration, the levels of BChE were inversely correlated with the rate of intake (positively related to the inter-infusion interval) during the last two sessions before sacrifice and blood collection (**Figure 28B**) ($N=20$, $r=0.4558$, $r^2=0.2078$, $p < 0.05$). This showed that the higher the BChE activity, the slower the intake.

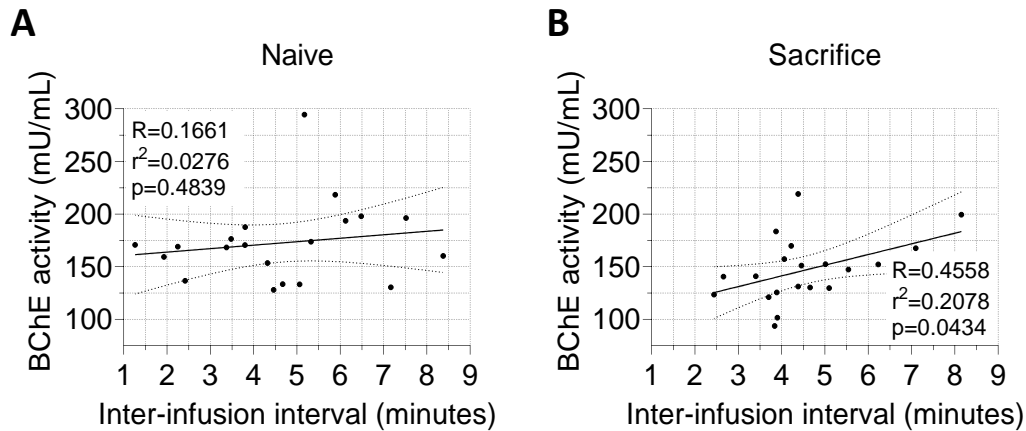


FIGURE 28: Relationship of BChE activity and inter-infusion interval- A. In the naïve condition, BChE activity was not associated with the average rate of intake in sessions 2 and 3. B. At sacrifice, higher BChE activity was associated with a longer average inter-infusion interval in the two sessions before sacrifice.

Butyrylcholinesterase activity and maladaptive seeking

Plasma BChE was then related to the average active no drug nose-pokes in the first drug period of the two sessions just prior to sacrifice to see if there was a relationship between maladaptive seeking and enzymatic efficiency. However, maladaptive seeking was not quantitatively related to BChE activity ($p=0.7924$) (**Figure 29**).

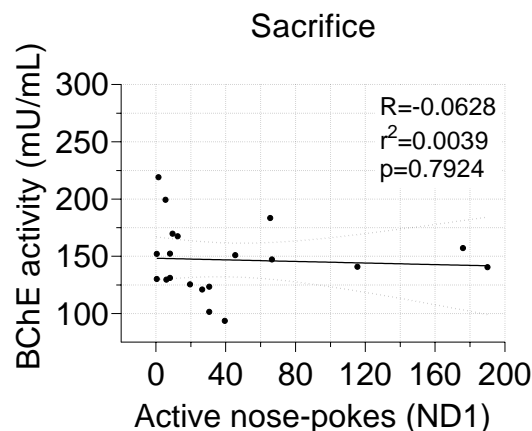


FIGURE 29: Relationship of BChE activity and maladaptive seeking patterns- BChE activity at sacrifice was not related to the number of average active nose-pokes in the first no drug period in the two sessions before sacrifice.

Additionally, when split in high and low maladaptive seekers, plasma BChE levels did not significantly differ between the two groups ($p=0.51$) (**Figure 30**).

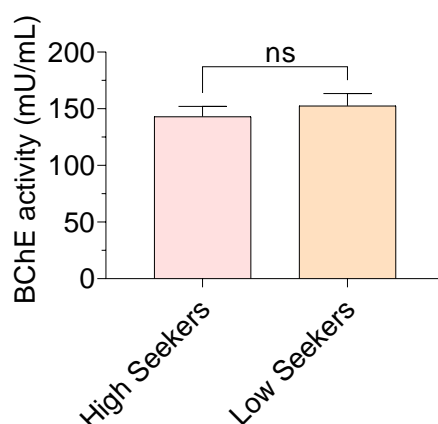


FIGURE 30: Relationship of BChE activity and maladaptive seeking group- BChE activity at sacrifice was not significantly different between high and low seekers ($p=0.51$).

DISCUSSION

Variations between brain structures in cocaine concentration

Despite identical cocaine administration before sacrifice, significant variation was observed in the amount of cocaine and metabolites found in the collected brain regions. While there is some evidence in the literature that cocaine concentration in the brain can differ between structures (Bystrowska et al., 2012), it is often overlooked through the use of a widely adopted mathematical model of brain cocaine levels (Pan et al., 1991; Zimmer et al., 2011). The model uses factors such as dose, the volume of the brain, time, and rate constants corresponding to the transfer from blood to brain, cocaine flow and cocaine elimination. Though it has been frequently used to relate cocaine pharmacokinetics to uncover important factors in developing translatable preclinical models of cocaine addiction, identification of high levels of variation in cocaine distribution as a function of drug exposure and behavior may limit the usefulness of this model. As the differences of cocaine concentration have been linked to self-administration behavior, to be more accurate, this model should be adapted to encompass potential changes in distribution as a function of individual behavioral variability.

Variations between individuals in cocaine concentration

The significant variation of intracerebral cocaine concentration between animals also needs further exploration. As this is the first time that it has been evidenced, there is a lot of work that needs to be performed to understand the mechanisms behind the observed differences. The individual variations are quite consistent between structures, suggesting that there is likely a mechanism affecting the whole brain, potentially dealing with transportation along the blood brain barrier.

The potential that differences in body weight could explain the difference in cocaine concentration observed was explored, however only an inverse correlation was found between one structure (the VTA) and the body weight of the animal, showing that this is likely not the explanation.

Individual variations in brain cocaine concentration and self-administration behavior

From there, cocaine concentration in each brain region was analyzed as a factor of self-administration behavior to see if the observed differences in cocaine concentration can explain variations in self-administration behavior. In most structures, the concentration of cocaine was inversely correlational to the number of injections taken in the self-administration session just prior to sacrifice. In all structures exhibiting the inverse correlation between the number of injections and cocaine concentration (excluding the BLA), there was also a negative correlation between the rate of intake and the cocaine concentration. The lower the cocaine concentration found in the brain, the faster the cocaine self-administration (measured by the inter-infusion interval). In summary, the less cocaine found in the brain after an identical dosing regimen, the faster and the more cocaine the individual normally self-administers.

These data suggest that the intracerebral cocaine distribution influences the rate of intake and amount of drug consumed. As mentioned previously, it has not been shown before and the exact mechanism allowing this to occur needs to be identified. There are multiple potential candidates that could be responsible for these individual differences in cocaine distribution found in the brain.

The first possibility is the recent identification of a proton-antiporter flux that is quantitatively much more important for cocaine's transport across the blood brain barrier than passive transport (Chapy et al., 2015). Differences in the activity of this proton-antiporter could be responsible for the differences in brain cocaine distribution

Secondly, the observed differences of cocaine and metabolites found in the brain could simply be due to altered metabolism of cocaine in these animals. A faster rate of intake and more drug consumption could simply emerge due to predisposed differences in metabolic efficiency of the pathways involved in the breakdown of cocaine. Rats with a fast cocaine metabolism would be able to take it faster, hence in higher amount. Another option is also that fast intake speeds and high intake levels can lead to changes in the metabolic pathways involved in the breakdown of cocaine and further promote the process.

However, it is unlikely that differences in cocaine intake are uniquely responsible for these individual differences in brain cocaine distribution. Although high seekers from this study had higher cocaine intake in acquisition, they did not differ significantly from low seekers for brain cocaine distribution nor for BChE activity.

Individual variations in brain cocaine concentration and cocaine metabolism

As the analytical technique permitted quantification of both cocaine and its metabolites, a deeper view of the efficiency of the metabolic pathways was explored. But first, validation of the analytical technique was performed by ensuring that the cocaine and metabolite concentrations were related. To do this, correlational analysis was done on each brain region between the cocaine concentration and EME, BZE, and norcocaine. The analysis confirmed that the cocaine concentration in most brain regions correlated with the amount of metabolites present, with the exception of BZE in the NacSh and ACC.

Further exploration of the ratio of cocaine and its metabolites were done by creating a ratio of each metabolite and the cocaine concentration. Consequently, analysis of the metabolite to cocaine ratio permits insight on the differences and efficiency of each of the different metabolic pathways within each brain region.

The relationship of norcocaine to cocaine, which depends on the CYP family of liver enzymes, does not seem to be related at all to rate of intake. The ratio of BZE to cocaine does not seem to be universally related to rate of intake nor amount of drug taken in previous self-administration sessions. Although this ratio is significantly correlated to rate and amount of cocaine intake in some brain regions, this relationship does not seem to be reliably upheld in multiple structures. This pathway is reliant on carboxylesterase activity, but as the ratio of cocaine to this metabolite is not reliably related to intake

behaviors, it is unlikely to be the factor explaining the difference in cocaine observed as a factor of addiction vulnerability.

However, in nearly every structure, the ratio of cocaine to EME was significantly or nearly significantly related to intake behaviors. The ratio of cocaine to EME was positively correlated to rate of intake and negatively correlated to number of injections. This suggests that the metabolic pathway producing EME from cocaine seems to be less efficient in animals that have slower intake rates and lower levels of intake. As this pathway is dependent on activity of the plasma cholinesterase BChE, the activity of this enzyme was hypothesized to be the reason behind the higher concentrations of intracerebral cocaine found in animals that had higher cocaine intake at faster rates.

Consequently, the next step was determination of individual differences of BChE in the plasma and exploration of how these differences of the enzyme relate to addiction vulnerability.

First, comparison of the BChE levels in rats before cocaine self-administration (naïve) and BChE levels in the same rats after cocaine self-administration (sacrifice) was done. We evidenced that BChE levels are significantly reduced after cocaine exposure, which is contradictory to another study using a chronic psychostimulant (methylphenidate- which like cocaine, acting on dopamine and norepinephrine reuptake transporters) administration that showed that chronic psychostimulant treatment in juvenile rats significantly increases BChE activity (Schmitz et al., 2012). However, the difference in results between these studies is likely due to the fact that blood collection at sacrifice in our study was done when the rats were 24 hours in withdrawal whereas in the study with methylphenidate, the blood collection occurred just after methylphenidate administration. As methylphenidate is solely metabolized in the liver by carboxylesterase 1, the alteration in BChE may simply be due to downstream cholinergic changes produced by the pharmacological mechanisms of methylphenidate. As there are limited studies that look at the concentration and activity of BChE in psychostimulant intoxication in withdrawal, it is highly possible that BChE activity increases during intoxication and decreases during periods of withdrawal.

One additional potential explanation for the difference in BChE activity before and after drug exposure can be because the blood was not collected in the same manner. During the naïve blood collection, the rats were anesthetized with ketamine and blood was

collected from the tail. During sacrifice blood collection, the rats were anesthetized with isoflurane and blood was collected from the trunk of the animal after decapitation.

There is, however, a gap in the literature when considering BChE and individual variations in addiction. Consequently, relating BChE to addiction vulnerability is a relatively novel exploration. There is evidence in humans that certain genetic variants of the BChE gene are associated with increased risk of crack cocaine use compared to intranasal cocaine use (Negrão et al., 2013). The polymorphism suggests that changes in functionality of BChE may possibly influence the preferred route of administration of the drug to compensate for the differences in metabolic efficiency of the enzyme. Therefore, it is possible that functional differences in this enzyme lead to differences in intake patterns.

Individual variations in BChE and cocaine self-administration behavior

To explore if differences in BChE levels before cocaine self-administration could explain the differences in intake, BChE levels were measured in blood samples before exposure to cocaine self-administration. However, the BChE levels were not related to intake patterns in the initial days of self-administration. Moreover, the BChE levels seemed to be more related to body weight, even though the relationship did not reach significance. However, at sacrifice, the BChE levels did not maintain the same relationship with body weight. BChE levels at sacrifice instead were positively correlated to rate of intake, meaning that the rats that take cocaine more quickly have lower BChE activity and those that take cocaine more slowly seem to have higher BChE activity.

Despite the results being the opposite of what was found in the brain, comparison between plasma levels of BChE and brain ratios of cocaine to metabolite are expected to be quite different. The quantification of cocaine and its metabolites from brain tissue may be a result of complete metabolism of cocaine within the brain, but there is also the possibility that the metabolites are produced in the plasma and are transported across the blood brain barrier, making the link between the cocaine and metabolite levels unclear.

This was also a surprising finding due to the fact that cocaine has been shown to weaken the blood brain barrier both by its direct effect on the brain microvascular endothelial cells as well as due to paracrine effects of drug-induced pro-inflammatory cytokines and chemokines on the blood brain barrier (Zhang et al., 1998). In vivo fluorescent

microscopy studies have also shown that cocaine injections lead to increased blood brain barrier permeability (Barr et al., 2020). Because of this, more frequent and higher drug intake would lead to higher levels of cocaine and metabolites in the brain, however clearly this was not the case.

Much more research needs to be performed to explain this discrepancy; however I believe it can be explained by exploring the functional differences of the recently discovered proton/cocaine-antiporter in animals resistant and vulnerable to cocaine addiction.

It should be noted however that there are significant limitations when considering exploration of the role of BChE in rodent models of cocaine addiction. While BChE plays a large role in the metabolism of cocaine in humans, it is found that this enzyme has a much smaller role in cocaine metabolism in rodents. Other than BChE, the other main enzyme contributing to cocaine metabolism in rats is carboxylesterase, which is not present in human blood (Li et al., 2005). While the results and exploration of the relationship between BChE activity and addiction vulnerability is interesting and justified due to the intracerebral ratio between EME and cocaine, it may not be completely translationally relevant due to inter-species differences in plasma esterase presence in rodents and humans. Future exploration of the influence of cocaine metabolism on addiction vulnerability in rodents can be explored by analyzing carboxylesterase activity in plasma to see its relationship with self-administration behavior and addiction vulnerability.

Individual variations in cocaine distribution/metabolism and maladaptive cocaine seeking

A quantitative relationship between cocaine concentration in the brain and maladaptive seeking during the no drug period was explored, however was not found to exist.

Interestingly a qualitative relationship was observed, which revealed that rats with low cocaine concentration in the brain after equal cocaine administration show a high trend to increase in maladaptive seeking in no drug periods from early (sessions 1-6) to later sessions (7-17). This data, while still preliminary, reaffirms the hypothesis that maladaptive seeking could be a downstream effect of variations in cocaine PK/distribution. Variations in PK/distribution would favor specific cocaine pharmacodynamics or a fast pattern of use, which would induce neurobiological adaptations promoting the development of maladaptive seeking. However, this would

not be the sole mechanism responsible for the vulnerability to develop a maladaptive seeking as high seekers only tended to show higher brain [cocaine] in the present study.

Conclusions

It has been widely known that altering pharmacokinetic dynamics leads to addiction-like behavioral alterations, eg increased response to cocaine incentive effects. However, here for the first time we show that variations in pharmacokinetic factors, such as distribution and metabolism, might indeed be sources of cocaine addiction vulnerability, as it has been widely shown for nicotine (Rubinstein et al., 2008). These differences may contribute to the altered influence of cocaine on adaptive inhibition in vulnerable animals that was shown in the previous chapter.

CHAPTER 3: PRELIMBIC CORRELATES OF MALADAPTIVE COCAINE SEEKING

INTRODUCTION

The medial prefrontal cortex (mPFC) has high involvement in a wide range of higher order behaviors that are fundamental for healthy neuropsychological function such as inhibitory control, cognitive flexibility, and interoception. Growing evidence place the dorsal part of the mPFC, ie the PL, and its projection to the NacC, at the core of adaptive operant behavior (Howland et al., 2022).

In the introduction, we presented data supporting that the amount of drug is not the critical factor for addiction, but that rather, repeated rapid rising and decay in brain cocaine levels, either imposed or self-administered, appear crucial. The data in chapter 2 support that individual variations in brain cocaine distribution may play a role in facilitating a fast pattern of use and may have an influence promoting a shift to maladaptive seeking during no drug periods.

Data support that the mPFC could be particularly sensitive to these cocaine PK factors.

Pharmacokinetics has been shown to have a potential influence on neurocognitive deficits because of drug use. Crack users and intranasal cocaine users were both found to have cognitive impairments compared to healthy controls, however crack users compared to intranasal cocaine users performed worse on prefrontal dependent measures including inhibitory control, eluding to a connection between the pharmacokinetics of the substance and prefrontal dependent impairments (Oliveira et al., 2018). Additionally, high frequency cocaine self-administration recruits the PL while low frequency self-administration does not (Belin et al., 2009; Martín-García et al., 2014).

The identification of potential differences in brain cocaine distribution in relation to addiction vulnerability (chapter 2) begs the question how the mPFC, and more specifically, the PL functioning is affected in the populations that express adaptive as compared to maladaptive seeking patterns.

For a real-time exploration of neural activity within the PL in relation to maladaptive seeking patterns we implemented fiber photometry with a genetically encoded fluorescent calcium indicator (GCaMP) during self-administration. Fiber photometry, a bulk fluorescent imaging tool, was chosen due to its versatility. Attesting of its reliability, since the creation of GCaMP around twenty years ago (TW Chen et al., 2013; Nakai et al., 2001), there have been a multitude of different fluorescent indicators such as dopamine (Patriarchi et al., 2018), acetylcholine (Borden

et al., 2020), serotonin (Unger et al., 2020), norepinephrine (Feng et al., 2019), glutamate (Marvin et al., 2013), orexin sensors (Duffet et al., 2022), and voltage or pH indicators (Knöpfel & Song, 2019; Miesenböck et al., 1998).

However, its use in combination with operant behavior is still rare, and the potential of coupling the two techniques is not fully exploited due to the difficulty in managing the many events that occur in operant sessions and their associations with calcium signals. We therefore created an open-source data analysis pipeline for fiber photometry coupled with operant behavior for optimal exploration of calcium transients in response to self-administration-associated events.

PL activity was analyzed as a function of maladaptive seeking patterns as well as levels of intoxication to uncover differences in PL functioning in addiction vulnerable vs resistant rats.

MATERIALS AND METHODS

SUBJECTS

Male CD Sprague Dawley rats (n=13) weighing 250-300g at their arrival were single housed in a temperature ($22 \pm 1^\circ\text{C}$) and humidity ($60 \pm 5\%$) controlled vivarium on a reverse-light cycle (ON 19h00, OFF 7h00). Food and water were provided *ad libitum*. Rats were handled daily before experimentation began.

SPECIFIC SURGERY

Stereotaxic- virus and fiber Implantation

After 3-6 days of habituation, the animals were anesthetized with isoflurane gas (5% induction, 2% maintenance) and placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). During surgery, body temperature was monitored and maintained at 37 degrees Celsius. Using a glass micropipette, 500 nL of GCaMP6f (AAV5.CaMKII.GCaMP6f- original titer: $2.30\text{E}+13$, final titer: $2.30\text{E}+12$, Addgene, Cambridge, MA, USA) was injected into the PL (AP: +3.0, ML: +/- 0.6, DV: -3.5 from skull) at a rate of approximately 250 nanoliters/minute. The micropipette was allowed to stay in place for 10 minutes to allow for diffusion. A hollow blunted tip 27G needle (outer diameter 410 micrometers) was lowered to the placement of the fiber then removed to prevent tissue accumulation under the recording area of the fiber. Lastly, the fiber (400

μm core, 0.48 NA, 5 mm in length; Doric Lenses, Quebec, Canada) was inserted and secured with Metabond (C&B Metabond, Parkell, Edgewood, NY, USA). The rats were treated post-op with the analgesic Metacam® (Boehringer Ingelheim, Germany) at a dose of 1 mg/kg, SC.

Intravenous catheterization

Four weeks after the stereotaxic surgery, the rats underwent intravenous catheterization under isoflurane anesthesia (as for the stereotaxic surgery). The catheterization procedure is described in the common methods.

FIBER PHOTOMETRY SYSTEM

Fiber photometry recordings were done with a 1-site Doric Lens photometry system with a calcium dependent (465 nm) and an isosbestic channel (405nm). Intensity of the 405 and 465 nm lights were sinusoidally modulated at 572 and 268 Hz respectively. Light was coupled to a filter cube (FMC4, Doric Lenses), converging onto a patch cord that was connected to the animal's implanted optical fiber. Fluorescence collection was through the same patch cord then passed to a photoreceiver (Visible Femtowatt Photoreceiver Module Model 2151, Newport). Output control and data acquisition was synchronized through a Doric Lens Photometry Console then passed to a PC that ran Doric Neuroscience Studio software. Data was low pass filtered with a cutoff frequency of 12 Hz.

COUPLING OF FIBER PHOTOMETRY AND SELF-ADMINISTRATION SYSTEMS

The Imetronic behavioral system (see common methods for a description of the self-administration apparatus) was connected to the Doric Lens fiber photometry system by a TTL output. This TTL output initiated and stopped the fiber photometry recordings. However, the behavioral data and fiber photometry were collected independently.

SPECIFIC INTRAVENOUS SELF-ADMINISTRATION PROCEDURE

Basal self-administration sessions

They were run as described in the common methods. In recorded self-administration sessions, fiber photometry recordings occurred for 4 minutes surrounding the switch from the first drug to the first no drug period (**Figure 31A**).

No drug pre (ND pre) session

The no drug pre session was identical to the no drug pre sessions in chapter 1 (see Materials and Methods).

However, in the recorded ND pre sessions, 6 of the 11 recorded sessions contained the ND pre switch from darkness (**Figure 31B**) whereas the others had a switch from the drug DS to the ND pre period (**Figure 31C**).

Non-contingent injection (NCI) baseline session

The non-contingent injection baseline session was a normal self-administration session with a 16-minute fiber photometry recording occurred (the last minute of the first drug period and all 15 minutes of the first no drug period) (**Figure 31D**).

Non-contingent injection (NCI) session

The NCI session was identical to the NCI baseline session, but with two non-contingent injections in the no drug period (as previously described in chapter 1) (**Figure 31E**).

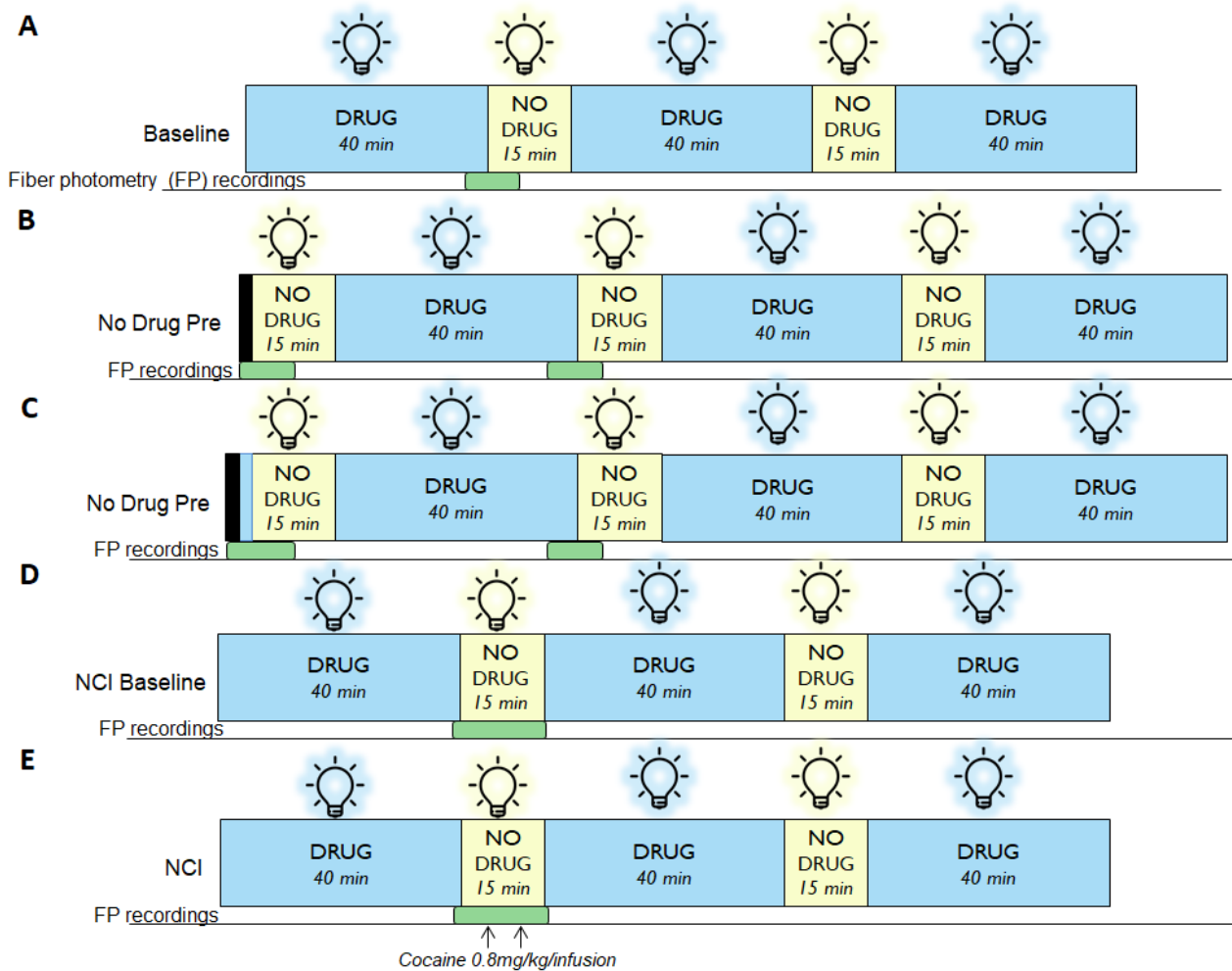


FIGURE 31: Fiber photometry recordings- A. In recorded self-administration sessions, fiber photometry recordings occurred for 4 minutes surrounding the switch from the first drug to the first no drug period. B. In 6/11 of the recordings used for ND pre analysis, the switch to the ND pre occurred from darkness. C. In the remaining sessions, the switch to the ND pre occurred after a short (10 second) presentation of the drug DS. The second recording (switch from the drug period to the intoxicated no drug period) remain unchanged in the two sessions. D. In the recorded NCI baseline session, the fiber photometry recording occurred for 16 minutes: from the last minute of the drug period to the end of the first no drug period. E. The NCI recording session was identical to the NCI baseline session, but with two non-contingent injections in the no drug period.

Fiber photometry self-administration session

The protocol was identical to the basal self-administration, however, during the recording sessions, the rats were connected to a dual pharmacology/optic fiber-motorized commutator (Imetronic, Marcheprime, France) which ensured that the patch cord and the line containing the cocaine solution rotated smoothly and in parallel to prevent tangling (Figure 32).

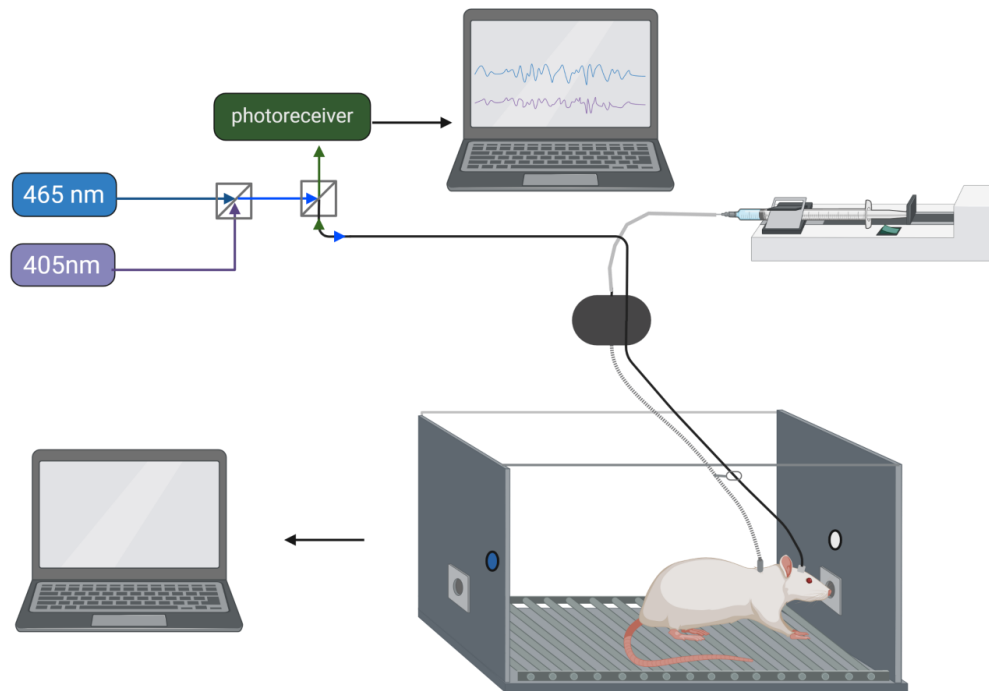


FIGURE 32: Coupling of fiber photometry with self-administration- During the recording sessions, the rats were connected to a dual pharmacology/optic fiber-motorized commutator which ensured that the patch cord and the line containing the cocaine solution rotated smoothly and in parallel to prevent tangling of the two lines. Fiber photometry recordings were initiated and stopped by a TTL pulse generated by the Imetronic behavioral system. Created with BioRender.com.

HISTOLOGY

At the end of the experiment, the rats were deeply anesthetized with an intraperitoneal injection of a mix of pentobarbital and lidocaine (200 mg/kg pentobarbital / 20 mg/kg lidocaine). The animals were transcardially perfused with 300 milliliters of 4% paraformaldehyde at a rate of 60mL/minute, and then their brains were removed and stored in 4% PFA. The brains were sliced (thickness 50 μ m) and immediately placed on glass slides. Proper fiber placement and adequate virus expression was analyzed using a Nikon Eclipse inverted microscope (Ti-U) (Figure 33A-B).

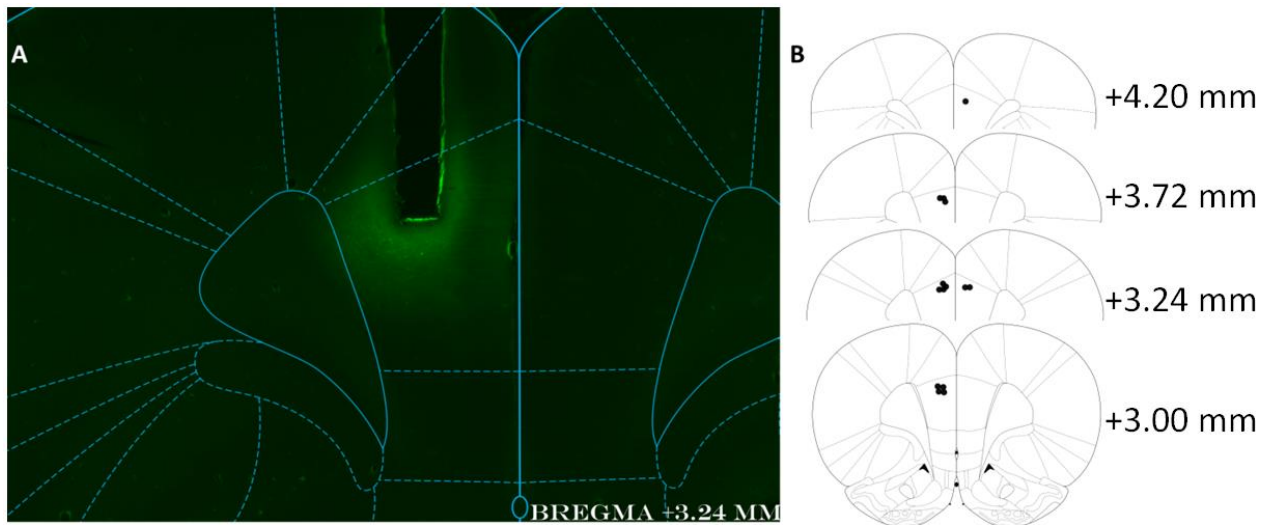


FIGURE 33: Histology- A. An example histological slice showing fiber placement and viral GCaMP expression. B. Locations of the fiber tip in all rats used for analysis.

EXPERIMENTAL TIMELINE

Rats were allowed to habituate to the vivarium for 3-6 days before stereotaxic surgery. Four weeks after the stereotaxic surgery, the rats underwent intravenous catheterization and started training for cocaine self-administration 5 days later.

Basal recorded self-administration sessions occurred between session 17 and 46 while recorded no drug pre sessions occurred from session 28 to 36. NCI baseline sessions occurred from session 38-45 while NCI sessions occurred from session 34-41 (Figure 34).

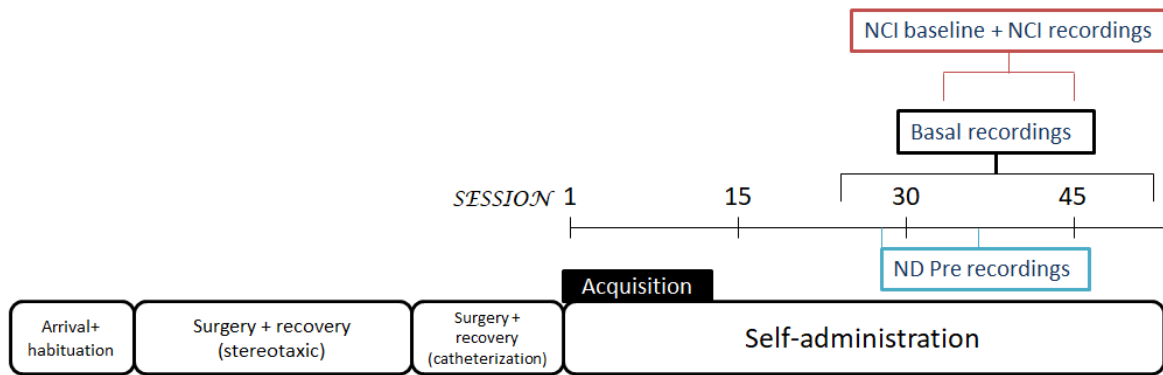


FIGURE 34: Experimental timeline: Chapter 3- In all experiments, rats had at least 16 sessions for acquisition. Basal self-administration recordings happened from session 17-46 while recorded ND pre sessions occurred from session 28-36. NCI baseline sessions occurred from session 38-45 while NCI sessions occurred from session 34-41.

SPECIFIC DATA AND STATISTICAL ANALYSIS

Intravenous self-administration

Acquisition of intravenous cocaine self-administration behavior was analyzed as described in the common methods.

Non-contingent injection baseline session

Variables of interest were: Active nose-pokes during the first 5 minutes and last ten minutes of the first no drug period and peak frequency and amplitude during the first 5 minutes and last ten minutes of the first no drug period

Active nose-pokes during the first five and last ten minutes of the non-contingent injection baseline session were compared by a paired t test.

Comparison of peak amplitude and frequency was done by paired t tests between peak frequency and amplitude within the first 5 minutes and last ten minutes of the no drug period.

Non-contingent injection session

Variables of interest were: Mean active nose-pokes during the first 5 minutes and last ten minutes of the first no drug period of the three sessions occurring the three days before the non-contingent injection session, active nose-pokes during the first 5 minutes and last ten minutes of the non-contingent injection session, as

well as peak frequency and amplitude during the first 5 minutes and last ten minutes of the NCI no drug period.

Active nose-pokes during the first five and last ten minutes of the non-contingent injection session were compared to the mean active nose-poking activity during the first five minutes and last ten minutes of three basal no drug periods using a repeated measures ANOVA with condition (baseline vs NCI session) and time (first 5 minutes vs last 10 minutes of NCI ND period) as within-subject factors, and maladaptive seeking group (high vs low) as between-subject factors when relevant. Significant main effects or interactions were explored by pairwise comparisons of means using the Fisher LSD post-hoc test.

Comparison of peak amplitude and frequency was done by paired t tests between peak frequency and amplitude within the first 5 minutes and last ten minutes of the no drug period.

ND Pre self-administration session

Variables of interest were: Mean active nose-pokes during the drug free ND period (ND pre) and the intoxicated ND period (ND1) of the recorded ND Pre sessions, average post-event Z score for the 3 seconds following the DS shift, as well as peak amplitude and frequency.

Mean active nose-pokes within the ND Pre sessions during the drug-free ND period (ND pre) and the intoxicated ND period (ND1) were compared using a repeated measures ANOVA with condition (ND pre or ND1) as a within-subject factor and maladaptive seeking group (high vs low) as between-subject factors when relevant. Significant main effects or interactions were explored by pairwise comparisons of means using the Fisher LSD post-hoc test.

Secondly, the average Z-score for three seconds following the presentation of the no drug discriminative stimulus was compared in the drug-free ND period (ND pre) and the intoxicated ND period (ND1) using a repeated measures ANOVA with condition (ND pre or ND1) as a within-subject factor and maladaptive seeking group (high vs low) as between-subject factors when relevant. Significant main effects or interactions were explored by pairwise comparisons of means using the Fisher LSD post-hoc test.

Comparison of peak amplitude and frequency was done by paired t tests between peak frequency and amplitude before and after the discriminative stimulus change.

Criteria for identification of High and Low maladaptive seekers

High and Low seekers rats were identified as described in the common methods.

Group comparisons

See common methods for self-administration behavior analysis.

Fiber photometry data and analysis

Pyfiber:

Fiber photometry analysis was performed using pyfiber, an open-source python library we developed that is aimed at facilitating the merge of operant behavior (using Imetronic interfaces) with fiber photometry data. This tool imports behavioral data files from Imetronic operant behavior boxes and FP recording data, and performs quick and flexible analyses of the calcium recordings in relation to behavioral responses or specific experimental events. See results.

PyPi:

<https://pypi.org/project/pyfiber/>

Read The Docs:

<https://pyfiber.readthedocs.io/en/latest/>

Github:

<https://github.com/matias-ceau/pyfiber>

Signal Processing- $\Delta F/F$:

Movement artifacts and artifacts due to photobleaching can be removed using an isosbestic channel, which does not contain calcium-dependent events. Signal processing ($\Delta F/F$) is done on the two channels to remove the influence of the artifacts from the resultant calcium dependent channel.

$\Delta F/F$ was performed by performing a linear regression on the isosbestic and calcium dependent channels with the least square polynomial fit of degree 1. The regression coefficients were then used to scale the isosbestic channel (control) to the calcium-dependent channel (signal) by using equation below. The resulting value corresponds to an activity ratio compared to the control channel.

$$\frac{\Delta F}{F_0} = \frac{\text{signal} - \text{fitted isosbestic}}{\text{fitted isosbestic}}$$

Peak Analysis:

Peaks in fiber photometry correspond to when a fluorescent signal is emitted from the fluorescent sensor. Peak analysis is performed to have a better understanding of the dynamics of the neural activity. Despite the fiber photometry signal not necessarily being perfectly synced with spiking activity, peak analysis from fiber photometry recordings can provide valuable insight into the changes in neural activity of the brain region of interest.

Peak analysis is performed on the $\Delta F/F$ trace with a default of 10 second windows to limit the impact of signal decay (caused by photo-bleaching) which leads to an underestimation of peaks towards the end of the recording. Then, inside each window, the baseline median absolute deviation (MAD, or bMAD) is calculated and a first threshold is set at 2.5 times the MAD. Signal data below this first threshold is removed and a new threshold is based on the MAD of the remaining data (pMAD, or peakMAD, set at 3.5 times this second MAD). All signal data higher than this second threshold are considered as transients. Transients correspond to groups of consecutive data points occurring close together due to the high recording sampling rate (1000 Hz). The final step is thus selecting timestamps for the highest point in each group of consecutive points to avoid falsely identifying multiple events (when it was a continuation of one fluorescent emission, or peak). From this, the peak frequency and amplitude can be calculated.

Perievent Analysis:

Perievent analysis is performed 3 seconds before and after the events of interest to see how bulk fluorescence changes are related to events. Perievent analysis permits an understanding of changes in population dynamics as a function of the event of interest.

Perievent analysis was performed by calculating Z-scores, using the time preceding the event as the baseline. The average perievent Z-score was then used in analysis.

$$Z_{peri} = \frac{\frac{\Delta F}{F_{0_{perievent}}} - \text{mean} \left(\frac{\Delta F}{F_{0_{baseline}}} \right)}{SD \left(\frac{\Delta F}{F_{0_{baseline}}} \right)}$$

RESULTS

PYFIBER DEVELOPMENT

Principle

As mentioned earlier, extracting the many different events occurring in multiple operant sessions and specifically relating them to calcium imaging is a challenge. During this PhD, I supervised Matias Ceau as a M1 and M2 intern whose expertise in Python coding gave me the opportunity to conceive a Python library that allows combining behavioral and FP data, and analyzing calcium activity in relation to various self-administration events, notably applied below to the response to the shift from drug to no drug periods. In this project, I oversaw the project management while Matias was in charge of the development.

The choice of a library (rather than an integrated application) was made to allow the greatest possible flexibility for integration in other information systems. As we will see with the example on the following pages, it can be used with a Jupyter Notebook (<https://docs.jupyter.org/en/latest/start/index.html>).

The library is based on three main modules: Behavior, Fiber and Analysis.

The Behavior module allows analysis of data from Imetronic .dat files either for a single file (Behavior) or for a batch of files (MultiBehavior). It is then possible to compare the raw data between animals and/or sessions.

The Fiber module allows extraction and normalization of the data from the fiber photometry recordings. It is then possible to visualize the evolution of the calcium signal over time and to retrieve the data in form of tables. Fiber allows

the calculation of transients, the visualization, and the export of data in csv format (df/F and Z-score over time - see 'Signal processing and analysis in the Methods section).

The Analysis module uses both the Behavior and Fiber modules. It analyzes calcium signals in relation to specific behavioral responses or scheduled events (eg shift in light condition) for one session (Session) or multiple sessions (MultiSession).

A configuration file in .yaml format (<https://en.wikipedia.org/wiki/YAML>) describes the different phases and events of a behavioral session: by modifying this file, it is possible to analyze any experiment that uses the Polymodal Imetric system. It also describes the structure of the data from the fiber photometry system, as well as all the events of interest used in the analyses [eg shift from Drug to ND periods, nose-pokes (NP) during time-out (TO)...].

Below is a summary of the different modules that are available with pyfiber. For full detail see <https://pyfiber.readthedocs.io/en/latest/>

Behavior module - behavior:

behavior = pyfiber.Behavior('path_to_the_dat_file')

Method	Feature	Command example
Figure	Plot data along time.	behavior.figure('inj1')
Summary	Show a graphical summary of main events and intervals (as defined in the config file).	behavior.summary()
Timestamps	Return timestamps of an event according to filters.	behavior.timestamps('inj1', interval = ('D_1'))
Export Timestamps	Same as Timestamps with a graphical representation.	behavior.export_timestamps('inj1', interval = ('D_1'))
Events	Return timestamps of events that occur when the TTL1 is 'ON'	behavior.event('inj1')
Intervals	Return list of all intervals defined in the configuration file.	behavior.intervals()
Dat visualisation	Return a dataframe summarizing the IMETRONIC data from the dat file as well as graphical representations.	behavior.what_data()
Specific Dat visualisation	Return a dataframe summarizing the IMETRONIC data from the dat file for a specific event.	behavior.get('INJ1')
Movement	Represent a heatmap of localization in the box (left or right).	behavior.movement()

Behavior module - multibehavior:

```
multibehavior = pyfiber.MultiBehavior('path_to_the_repertory_with_dat_files')
```

<u>Method</u>	<u>Feature</u>	<u>Command example</u>
Count	Return a dataframe with number of an event cumulated per second.	multibehavior.count('np1')
Cumul	Show a graph with events cumulated over time animal per animal.	multibehavior.cumul('np1')
Show Rate	Show rate of selected events for all sessions, with the option of selecting desired percentiles of the individuals	multibehavior.show_rate('np1')
Summary	Same as summary of Behavior module but for all the analyzed sessions.	multibehavior.summary()

Fiber module:

```
fiber = pyfiber.Fiber('path_to_the_csv_file')
```

<u>Method</u>	<u>Feature</u>	<u>Command example</u>
Plot	Show graphs with Fiber data.	fiber.plot()
Plot transients	Show a graph with transients among time.	fiber.plot_transients()
Export to CSV	Export data to a CSV file.	fiber.to_csv('name.csv')
Get	Extracts data array for a specific column of a recording.	fiber.get('signal')
Normalize	Normalize data with specified method.	fiber.norm(method='F',rec=1)
Peaks	Return a dictionary containing a data frame with peak data (timestamps, dF/F, zscore) for all recordings.	fiber.peaks

Analysis module:

```
session = pyfiber.Session('path_to_the_folder_with_dat_and_csv_file')
```

<u>Method</u>	<u>Feature</u>	<u>Command example</u>
Events	same as pyfiber.Behavior.events	
Intervals	same as pyfiber.Behavior.intervals	
Analyze	Prepare analyzed fiber data related to defined perievent window. Need to put it in a variable and then used it to get graphs or dataframes.	objAnalyze = session.analyze('np_effective', window=(1,1))
Plot	Plot data from the analyzed object.	objAnalyze.plot()
Update window	Update the window.	objAnalyze.update_window(window=(5,5))

```
multisession = pyfiber.MultiSession('path_to_the_folder_containing_session_folders')
```

Method	Feature	Command example
Analyze	Prepare analyzed fiber data related to defined perievent window. Need to put it in a variable and then used it to get graphs or dataframes.	objAnalyze = multisession.analyze('np_effective', window=(1,1))
Plot	Plot data from the analyzed object.	objAnalyze.plot()
Data	Return a dataframe with data.	objAnalyze.data
Update window	Update the window.	objAnalyze.update_window(window=(5,5))
Variables	Multiple variables of interest can be obtained (see full list here)	objAnalyze.preAVG_Z
Compare behavior	Compare behavior between individuals for a selected event. A graphical representation is outputted	Multisession.compare_behavior('np1')

Any dataframes can be exported to a .csv file by calling the command:
 'dataframe.to_csv('path to where you want to store the dataframe')

Example of application

Below, an example outline of how to use pyfiber is performed using Jupyter Notebook. The first step is importing pyfiber to Jupyter Notebook. This can be done after installing pyfiber in the anaconda prompt.

Then, to analyze multiple sessions at the same time, you would use MultiSession: you indicate the path to the folder that contains the subfolders which contain the individual's .dat (behavioral) and .csv (fiber) data files. In this example, a folder containing two subfolders: each containing a fiber and behavioral data file, with the folder name containing a title that includes information about the rat, experiment, and recorded self-administration session. Assign a name to this created variable.

To do perievent analysis, you call the variable created in the previous step (shown here as 'SingleRecording') and using the analyze function (SingleRecording.analyze()), you indicate the event of interest (shown here as the switch from the drug period to the no drug period ('switch_1_D')) and the window (in seconds) you want to use for the perievent analysis (shown here as 5,5- 5 seconds pre-event, 5 seconds post-event). Assign this as a new 'perievent' variable (shown here as 'DtoNDSwitch').

The perievent analysis can be shown by calling 'DtoNDSwitch.data'. This will show a table containing the recordings used, the time of the event, normalization method,

pre/post AUC, pre/post Z score, pre/post robust Z score, pre/post peak amplitude and frequency.

The perievent signal can be plotted by calling 'DtoNDSwitch.plot()', indicating which signal you want to be plotted. Shown here, the $\Delta F/F$ traces and robust Z-scores are plotted ('signal', and 'rob_zscores', respectively). The average of the recordings is plotted in black, while the individual recordings are plotted in red and blue.

```
In [3]: #here you are importing pyfiber. this is assuming that you already entered "pip install pyfiber" on your anaconda prompt
import pyfiber as pf
```

```
In [36]: #now, you are uploading multiple sessions, folder is one rat/recording containing the .dat imetric and .csv fiber data
SingleRecording=pf.MultiSession('C:/Users/dconlisk/Desktop/T')
```

```
21:31:19 ---
Importing folder AL21R_rat_9_SA_j26...
21:31:19 --- IMPORTING C:/Users/dconlisk/Desktop/T\AL21R_rat_9_SA_j26\bsa16112021c20_03.dat...
21:31:19 --- Detecting hled_on... ['conditional', [1, 1], ['_P', 1]]
21:31:19 --- Detecting hled_off... ['conditional', [1, 1], ['_P', 0]]
21:31:19 --- Detecting led1_on... ['conditional', [1, 2], ['_P', 1]]
21:31:19 --- Detecting led1_off... ['conditional', [1, 2], ['_P', 0]]
21:31:19 --- Detecting led2_on... ['conditional', [1, 3], ['_P', 1]]
21:31:19 --- Detecting led2_off... ['conditional', [1, 3], ['_P', 0]]
21:31:19 --- Detecting np1... ['conditional', [3, 1], ['_V', 1]]
21:31:19 --- Detecting np2... ['conditional', [3, 2], ['_V', 1]]
21:31:19 --- Detecting inj1... ['conditional', [6, 1], ['_L', 1]]
21:31:19 --- Detecting tt11_on... ['conditional', [15, 1], ['_L', 1]]
21:31:19 --- Detecting tt11_off... ['conditional', [15, 1], ['_L', 0]]
21:31:19 --- Detecting x_coordinates... ['simple', [9, 1], ['_X']]
21:31:19 --- Detecting y_coordinates... ['simple', [9, 1], ['_Y']]
21:31:19 --- Detecting xy_timestamps... ['simple', [9, 1], ['TIME']]
21:31:19 --- Detecting HLED... ['ON_OFF', 'both', ['hled_on', 'hled_off']]
21:31:19 --- Detecting LED1... ['ON_OFF', 'both', ['led1_on', 'led1_off']]
21:31:19 --- Detecting LED2... ['ON_OFF', 'both', ['led2_on', 'led2_off']]
```

```
In [49]: #Now you will do perievent analysis on the D to ND switch with a window of 5 pre / 5 post
DtoNDSwitch=SingleRecording.analyze('switch_1_D', window=(5,5))
```

```
21:41:34 --- Event timestamps: [2400.001]
21:41:34 --- Chosen interval: [(0.0, 9000.01)] (any)
21:41:34 --- Normalizing recording 2 with method 'F'
21:41:34 --- Normalizing recording 2 with method 'raw'
21:41:34 --- Event timestamps: [2400.001]
21:41:34 --- Chosen interval: [(0.006, 9000.025)] (any)
21:41:34 --- Normalizing recording 2 with method 'F'
21:41:34 --- Normalizing recording 2 with method 'raw'
```

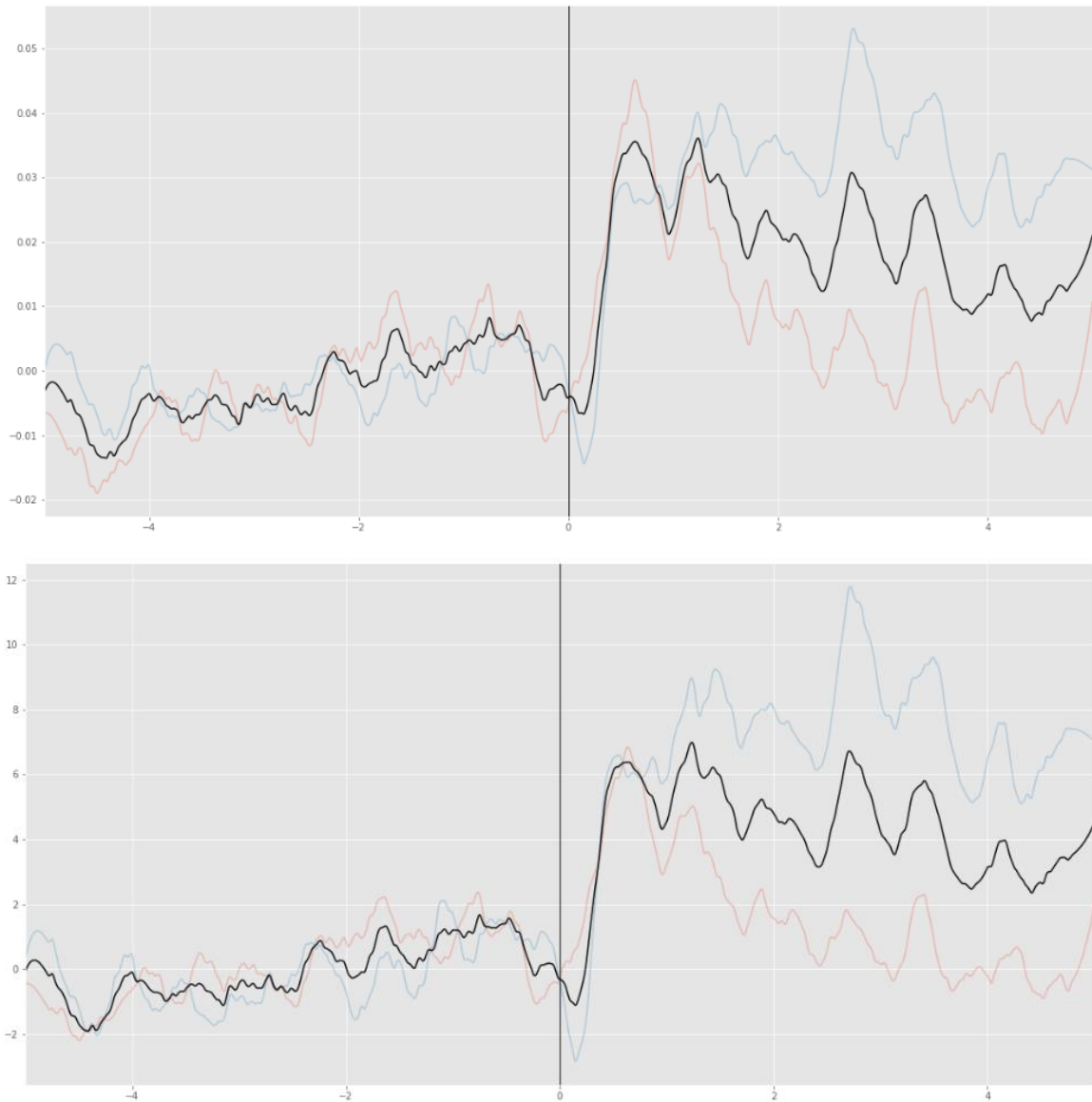
In [50]: #doing this shows you the data of interest that was analyzed with all the analyzed averages
DtonSwitch.data

Out[50]:

	behaviorfile	event_time	fiberfile	normalisation	postAUC	pos
AL21R_rat_9_SA_j26	C:/Users/dconlisk/Desktop/T\AL21R_rat_9_SA_j26...	2400.001	C:/Users/dconlisk/Desktop/T\AL21R_rat_9_SA_j26...	F	0.040050	
AS21R_rat_12_SA6_j36	C:/Users/dconlisk/Desktop/T\AS21R_rat_12_SA6_j...	2400.001	C:/Users/dconlisk/Desktop/T\AS21R_rat_12_SA6_j...	F	0.150752	

2 rows x 31 columns

In [55]: #You can then plot the perievent analysis, chosing your method of choice. I included the first processed signal (deltaF/F)
#and the robust Z score
DtonSwitch.plot('signal'), DtonSwitch.plot('rob_zscores')



PRELIMBIC ACTIVITY AND MALADAPTIVE SEEKING EXPRESSION

Acquisition of cocaine self-administration

Rats acquired cocaine self-administration as shown by both the significant discrimination between the active and inactive holes [ANOVA, hole effect: $F(1, 12)=12.571$, $p<0.005$] with a trend to increase over sessions [hole x session effect: $F(15, 180)=1.6599$, $p=0.06265$] and an increase in the number of cocaine injections per session which achieved stability in the population by day 9 [session effect, $F(15, 180)=9.5584$, $p<0.00001$].

Regarding no drug periods, we analyzed here the first one (as this is the one that will be used for rats' classification as high and low maladaptive seekers). In this period, a higher number of responding in the active hole was also observed [ANOVA, hole effect: $F(1, 10)=31.544$, $p<0.0001$] with fluctuation over the sessions [hole x session effect: $F(15, 840)=2.686$, $p<0.0005$].

Manipulation 1: PL recruitment by an adaptive inhibitory discriminative stimulus (no drug DS)- relationship with active no drug responding

The relationship between the extent of recruitment of the PL and maladaptive seeking patterns were explored through the analysis of the recorded basal self-administration sessions.

A correlation was performed for each recording with the average z-score post-switch (from the first drug period to the first no drug period) plotted against the latency to the first active nose-poke that occurred during the no drug period. Stronger calcium activity in response to no drug period onset correlated with an increased latency to initiate maladaptive seeking behavior [$N=45$, $r^2 = 0.3368$, $r = 0.5804$, $p < 00005$] (**Figure 35A**), indicating that the less the PL is recruited in response to the ND DS, the sooner the maladaptive seeking behavior is reinitiated in the no drug period.

Additional correlations were performed plotting the average z-score post-switch (from the first drug period to the first no drug period) against the total number of active nose-pokes during the consequential no drug period. There was a tendency for the PL recruitment by the ND DS to be correlated with total active nose-pokes during the ND period, suggesting that the more the less the PL is recruited, the more maladaptive seeking is expressed (**Figure 35B**). Removal of an extreme rat that expressed highly

maladaptive seeking behavior during the no drug period (between 900-1300 active nose-pokes within the 15-minute drug period) led to observe a significant inverse correlation between the number of active nose-pokes in the no drug period and the recruitment of the PL (**Figure 35C**) (N=43, $r^2 = 0.2058$, $r = -0.4536$, $p < 0.01$). Additional recordings with more intermediate phenotypes will probably reinforce this negative relationship, which together with the significant correlation between PL recruitment by the ND DS and latency to first nose-poke support that poor PL recruitment contribute to maladaptive seeking.

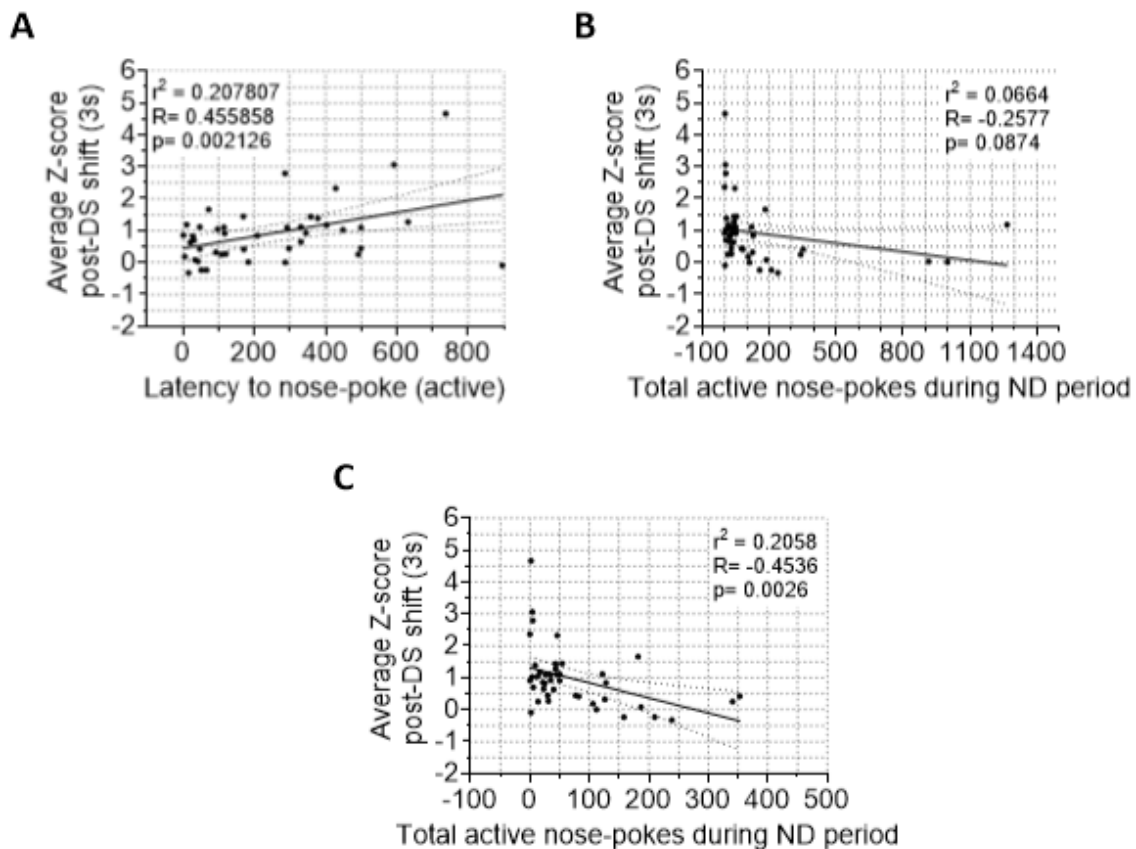


FIGURE 35: PL recruitment in response to presentation of the no drug DS and maladaptive seeking patterns- A. There was a correlation between the average z-score (3s) after presentation of the ND DS and the latency to the first active nose-poke in the no drug period. B. There was a tendency that the average z-score (3s) after presentation of the ND DS was also correlated to the total number of active nose-pokes in the no drug period, however it was not significant ($p=0.0874$). C. Removing an extreme rat that was nose-poking between 900-1300 times in the 15 minute no drug period led to significance of the correlation between the average PL recruitment (3s) in response to the ND DS and the number of active nose-pokes during the signaled no drug period.

To control for the potential bias that the amount of cocaine intake or the rate of intake in the previous drug period influences the recruitment of the PL, a correlation was performed for each recording with the average z-score post-switch plotted against the number of injections (**Figure 36A**) and the inter-infusion interval (**Figure 36B**) in the previous drug period. Though there was a trend indicating that the more injections during the previous drug period, the less the PL is recruited, it did not reach significance [N=45, $r^2 = 0.0622$, $r = -0.2494$, $p = 0.0985$]. The relationship became less significant when looking at the rate of intake [N=45, $r^2 = 0.0517$, $r = 0.2276$, $p = 0.1327$].

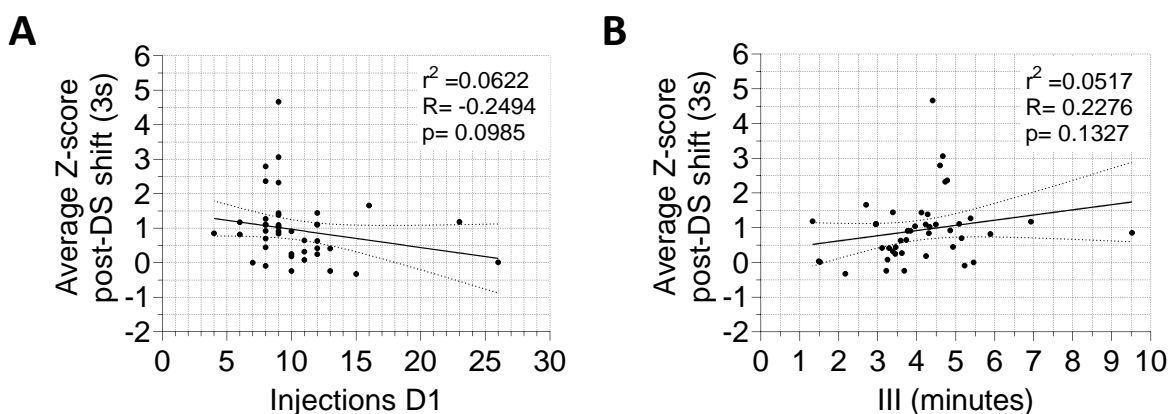


FIGURE 36: PL recruitment in response rate of intake and number of injections- A. There was a tendency, however no correlation between the average z-score (3s) after presentation of the ND DS and the number of cocaine injections obtained in the drug period just prior to the recorded onset of the ND period. B. There was a tendency, however no correlation between the average z-score (3s) after presentation of the ND DS and the rate of cocaine intake in the drug period just prior to the recorded onset of the ND period.

As a final control exploring the extent of intoxication influencing PL recruitment in response to the presentation of the no drug DS light, correlational analysis was performed between the time from the last injection before the switch to the ND DS and the average Z score after the ND DS presentation (3 seconds). No correlation was found to exist, indicating that the level of recruitment of the PL in response to the ND DS is not highly controlled by the precise state of intoxication [N=45, $r^2 = 0.0022$, $r = 0.0468$, $p = 0.7602$] (**Figure 37**).

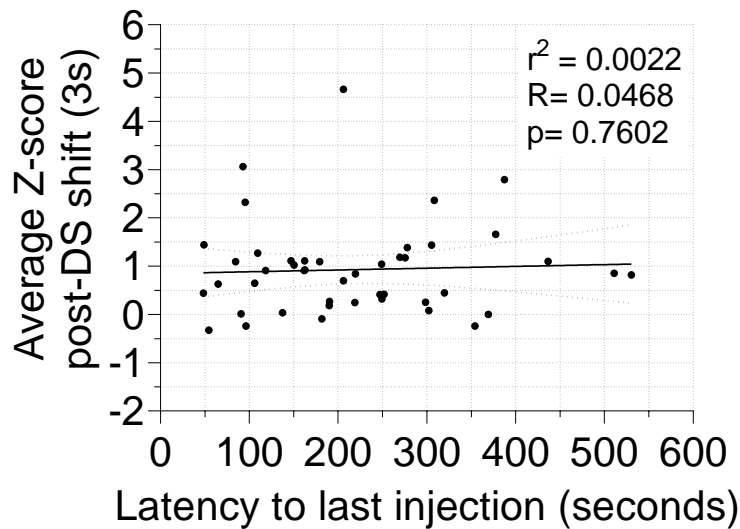


FIGURE 37: PL recruitment in response rate of intake and number of injections- There was no correlation between the average z-score (3s) after presentation of the ND DS and the latency to the last injection before the recorded onset of the ND period.

Manipulation 2: PL recruitment by an adaptive inhibitory discriminative stimulus (no drug DS)- relationship with active no drug responding as a function of drug state

Having shown that there is a link between PL recruitment by ND DS and initiation of maladaptive seeking in the no drug period, we studied the influence of the intoxication status on this relationship by comparing the recruitment of the PL by the ND DS after a drug period (no drug period following a first drug period, as in the previous paragraph) to the one in a drug free state (no drug period at the beginning of the session ND pre session) within the same sessions (**Figure 31B-C**). We related PL activity to behavior during the corresponding no drug periods.

Regarding ND pre sessions, 6 recordings were performed with presentation of the ND DS from darkness and 5 recordings were performed with the ND DS being presented after a short ten second presentation of the drug DS. As the perievent analysis uses the baseline pre-shift to normalize the signal, controls were performed to explore if this baseline period can be responsible for differences in perievent signals. The peak amplitudes and frequency during the 5 second baseline period before the switch were calculated, and it was found that neither the peak amplitude nor frequency differed between the two

conditions (frequency: $p=0.1128$; amplitude: $p=0.9639$) (**Figure 38A-B**). We therefore pooled the 11 ND pre periods.

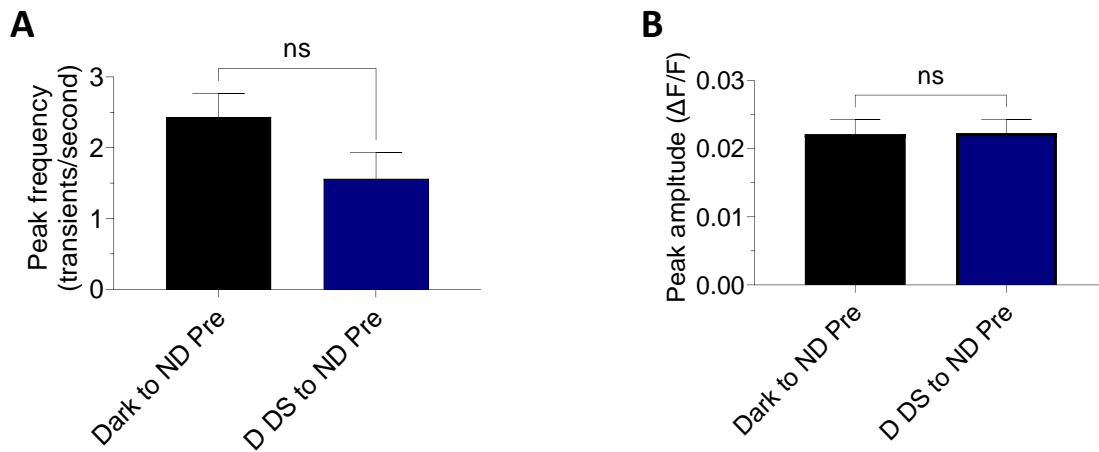


FIGURE 38: Peak frequency/amplitude analysis for ND pre sessions- A. Peak frequency and B. peak amplitude did not significantly differ in the baseline period before the switch to the ND pre period.

At the population level, the recruitment of the PL by the ND DS did not differ between the drug free and the intoxicated state ($p=0.9282$, ns) (**Figure 39A**), despite the fact that seeking during no drug differed significantly across all animals between the no drug pre to the intoxicated no drug period ($p<0.05$) (**Figure 39B**). In the next step, we analyzed whether the PL recruitment in these two conditions differed as a function of individual variations in expression of maladaptive seeking in the intoxicated state (the early marker of addiction vulnerability).

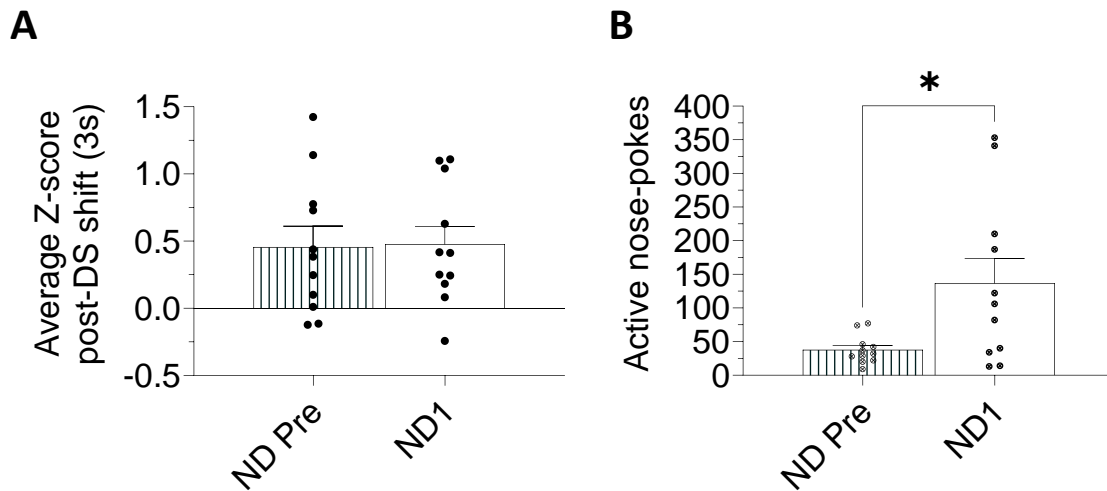


FIGURE 39: PL recruitment and behavior in drug free ND period vs intoxicated ND period- A. At the population level, the recruitment of the PL by the ND DS did not differ between the drug free and the intoxicated state ($p=0.9282$, ns) despite the fact that B. seeking behavior during no drug differed significantly across all animals between the no drug pre to the intoxicated no drug period. * $p<0.05$

PL cortex recruitment to no drug DS as a function of maladaptive seeking expression

Group analysis

To explore this, rats were classified as low or high seekers during no drug periods as described in the methods (based on a median split of the total active responding in the intoxicated no drug 1 period during the recorded ND Pre sessions), and their corresponding behavior during the ND pre sessions was analyzed (**Figure 40A**).

While obviously differing during the intoxicated no drug 1 period on which they have been classified, the two groups did not differ in no drug pre [$N=11$, ANOVA, maladaptive group effect: $F(1, 9)=22.543$, $p<0.005$; condition effect: $F(1, 9)=24.745$, $p<0.001$; maladaptive group x condition interaction: $F(1, 9)=14.825$, $p<0.005$], as observed in chapter 1. ND1 was associated with an increase in cocaine seeking only in the high rats ($p<0.0005$).

Notably high and low rats did not differ for the total number of infusions nor the mean inter-infusion interval during the drug period preceding the intoxicated ND (injections: $p=0.078405$; III: $p=0.1176$) (**Figure 40B-C**).

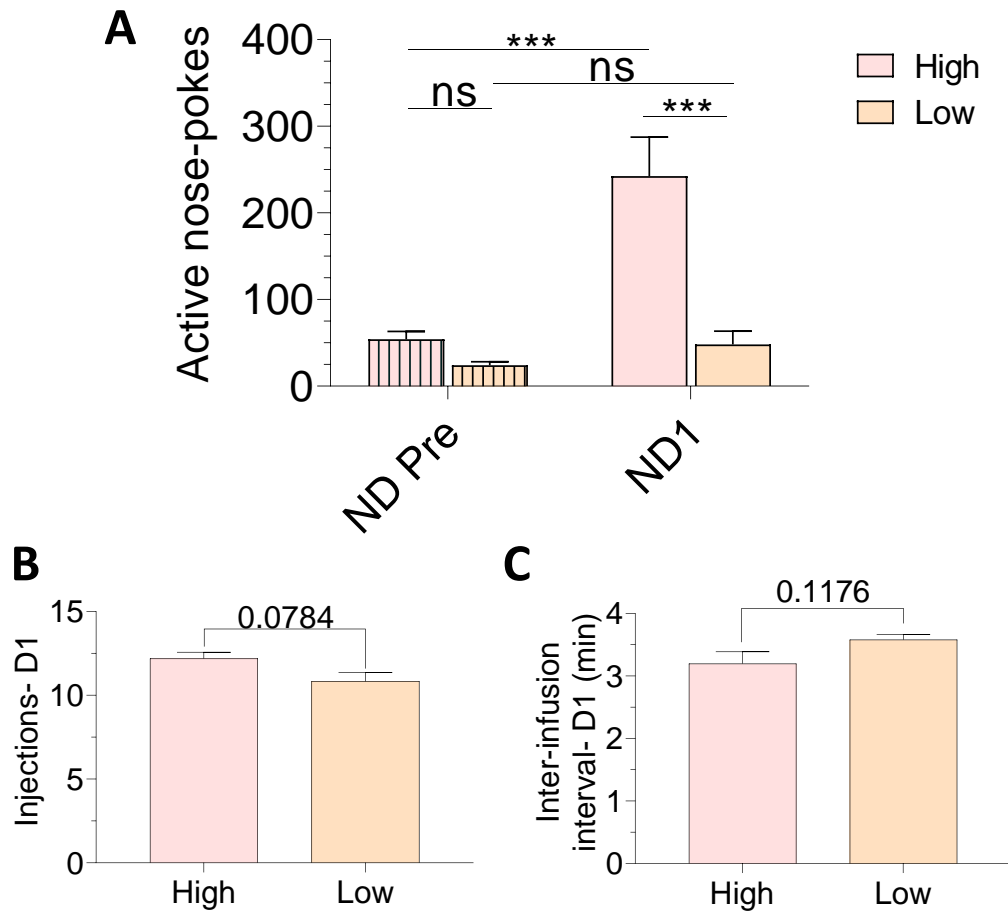


FIGURE 40: Behavior in drug free ND period vs intoxicated ND period as a function of vulnerability- A. High and low groups did not differ in active nose-pokes in the ND pre periods, while significantly differing in the intoxicated ND period. Additionally, the low group did not significantly differ in active nose-pokes between the drug free and the intoxicated ND periods while the high rats increased active nose-pokes significantly in the intoxicated no drug period compared to the drug free ND period. However, the high and low groups did not differ in B. injections or C. inter-infusion interval in the drug period between the two no drug periods. *** $p < 0.001$

Differences in recruitment of the PL in response to the no drug DS were then explored as a function of maladaptive seeking expression and cocaine status.

The two groups did not differ significantly over the two conditions [N=11, maladaptive group effect: $F(1, 9)=2.0369$, $p=0.18729$] nor as a function of the tested condition (ND pre vs intoxicated ND1) [maladaptive group x condition effect: $F(1, 9)=1.6468$,

p=0.23146]. Though it did not reach significance (probably due to limited number of rats) there was a tendency for the low animals to have higher recruitment of the PL compared to the high animals when presented with the ND DS in the intoxicated state (Figure 41). Also, the ND DS tended to recruit the PL more in the intoxication state than the drug free state in low rats while the opposite tendency (less recruitment in the intoxicated state than the drug free state) was observed for the high rats.

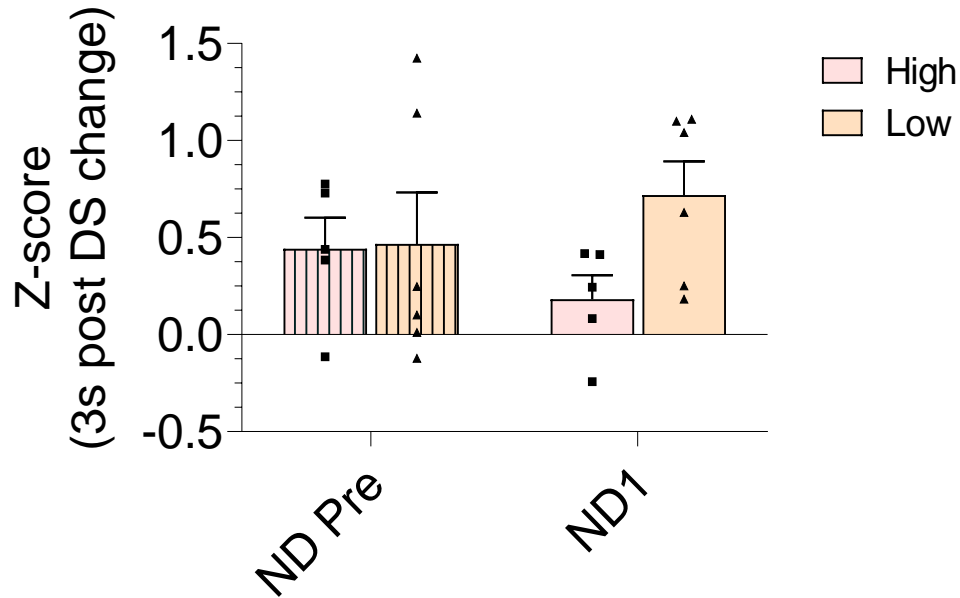


FIGURE 41: PL recruitment in drug free ND period vs intoxicated ND period as a function of vulnerability- A. The two groups did not differ significantly over the two conditions [N=11, group effect: $F(1, 9)=2.0369$, $p=0.18729$] nor as a function of the tested condition (ND pre vs intoxicated ND1) [maladaptive group x condition effect: $F(1, 9)=1.6468$, $p=0.23146$]. Though it did not reach significance (probably due to limited number of rats) there was a tendency for the low animals to have higher recruitment of the PL compared to the high animals when presented with the ND DS in the intoxicated state. The high animals seemed to recruit the PL more in a drug free state compared to an intoxicated state, while the low animals seemed to recruit the PL more in an intoxicated state as compared to a drug free state.

As a complement to the group analysis, we performed correlation analysis. We studied the relationships between PL recruitment by the ND DS in the intoxicated state and the latency to re-initiate seeking during this ND period, as well as the number of active nose-pokes during this ND period. In both the drug free (no drug pre) and the intoxicated no drug period, there was no correlation between the latency to the first active nose-poke

and the extent of recruitment of the PL (**Figure 42A-B**) (drug free: $p=0.99$, intoxicated: $p=0.47$). The limited number of recordings in these specific sessions probably explains why we were not able to find the correlation we had evidenced in the previous paragraph between recruitment of the PL by the ND DS and latency to reinitiate seeking in the intoxicated no drug period.

In the no drug pre, there was also no correlation between the total number of active nose-pokes in the no drug period and the extent of recruitment of the PL ($p=0.69$) (**Figure 42C**), while in the intoxicated no drug period, this relationship approached significance ($p=0.053$) (**Figure 42D**).

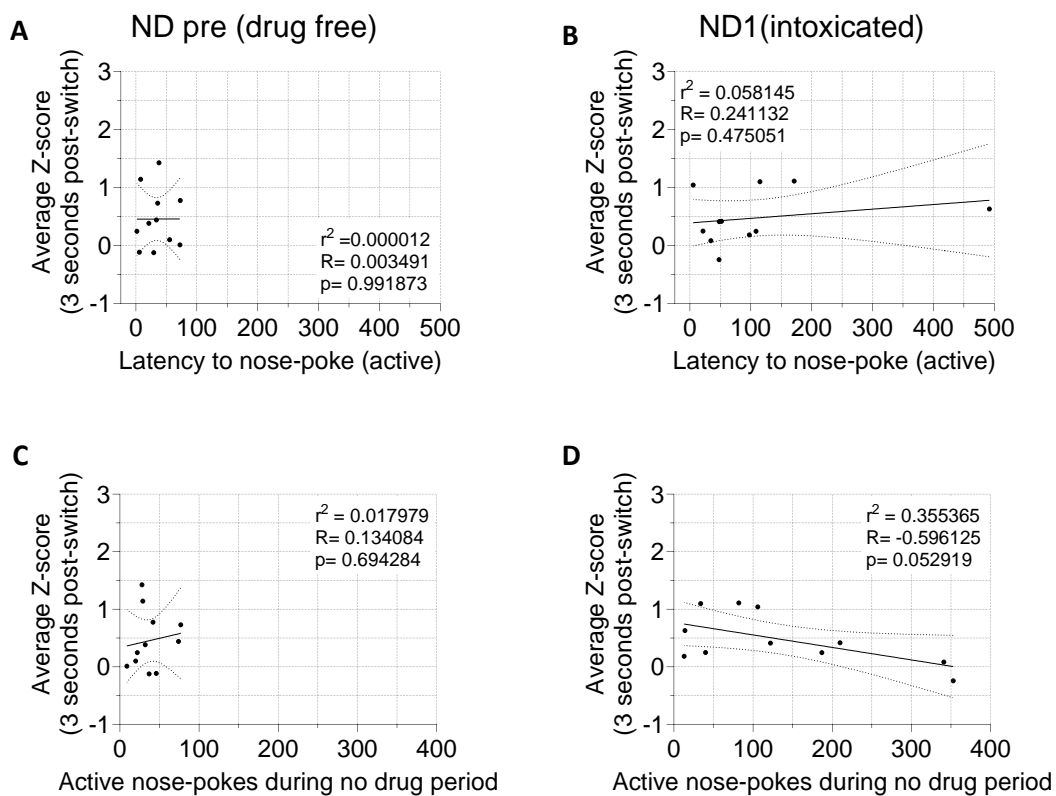


FIGURE 42: PL recruitment in drug free ND period vs intoxicated ND period related to maladaptive seeking- A. In the drug free ND period, recruitment of the PL was not correlated to the latency to the first active nose-poke nor C. the total active nose-pokes over the no drug period. B. In the intoxicated ND period, the recruitment of the PL was not correlated to the latency to the first active nose-poke. D. There was a trend that the recruitment of the PL was inversely related to the number of active nose-pokes in the no drug period.

Manipulation 3: PL activity during no drug period as a function of active no drug responding

Baseline Recordings over whole intoxicated ND periods

Within the recorded no drug periods (n=4), there was a significant difference in seeking activity within the first 5 minutes of the no drug period and the last ten minutes of the no drug period (**Figure 43A**). Due to this difference, it could be concluded that there was an initiation of maladaptive seeking patterns selectively in the last ten minutes of the no drug period. Consequently, analysis of the fiber photometry recordings was done by comparing the peak amplitude and frequency between the first 5 minutes and the last 10 minutes of the no drug period. There was a trend that the peak amplitude increased when comparing the two time points, however it did not reach significance (p=0.0761) (**Figure 43B**). The frequency of the peaks did not differ within these two time points however (**Figure 43C**).

From this, it seems that the occurrence of maladaptive seeking behavior is associated with an increase in the amplitude of the calcium peaks, while not significantly changing the frequency of the peaks.

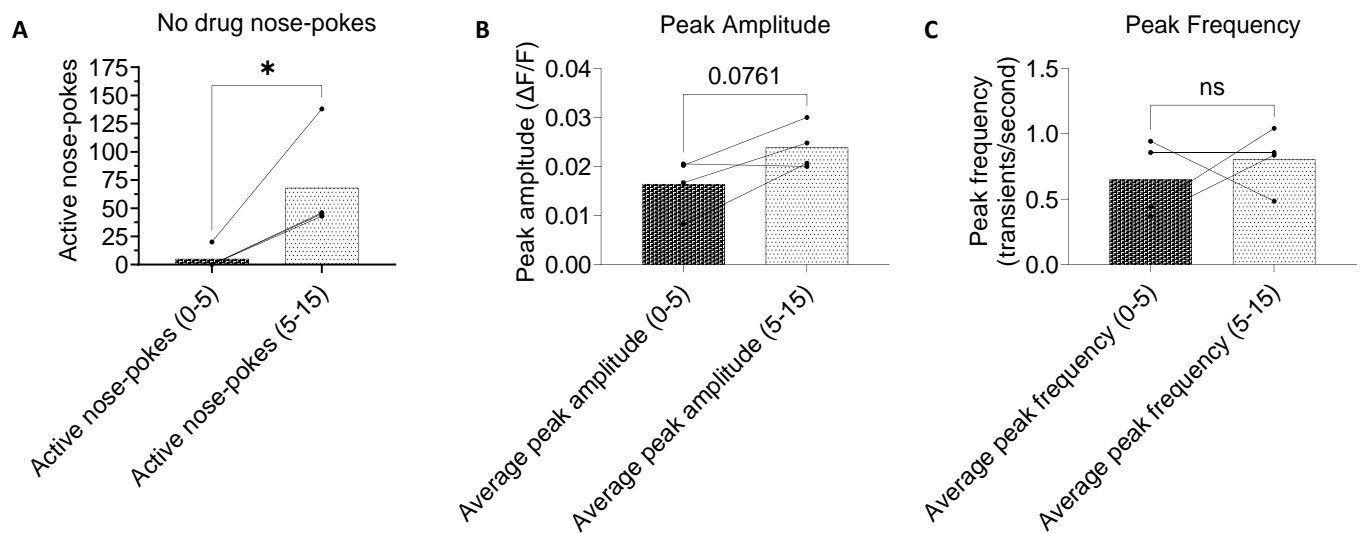


FIGURE 43: NCI Baseline- A. Rats had significantly different behavior in the first 5 minutes compared to the last 10 minutes of the no drug period. B. There was a trend that the peak amplitude increased from the first five minutes to the last ten minutes of the no drug period, however it did not reach significance. C. The peak frequency did not significantly differ between the first five minutes and last ten minutes of the no drug period.

Manipulation 4: PL activity during no drug period as a function of drug state- effect of non-contingent cocaine injections during no drug period

Based on the results of chapter 1 showing that non contingent injections of cocaine (NCI) during no drug period prevent re-initiation of maladaptive seeking, we therefore explored whether the PL activity (as a function of transient peak and amplitude) was related to this NCI effect. For this we performed the same type of recordings as described in the previous paragraph but applying cocaine NCI during the no drug period.

When comparing seeking patterns in the NCI session to previous no drug periods, there was no significant effect of condition (NCI or previous baseline activity) (condition effect: $F(1, 4)=3.2286, p=0.14678$) nor condition x time (first 5/last 10 minutes of ND period) ($F(1, 4)=3.6961, p=0.12690$). However, this is likely due to the small sample size of recordings obtained in NCI sessions ($n=5$). However, the trends indicate that despite the small sample size, the NCI was able to reduce seeking activity when compared to average active nose-pokes in the last ten minutes of the three previous self-administration sessions (**Figure 44A**). Additionally, contrary to what was previously observed in the baseline NCI sessions, there was no significant difference in seeking patterns in the first 5 and last ten minutes of the NCI no drug period (**Figure 44B**). Subsequent peak analysis showed that the NCI resulted in significantly reduced average peak amplitude between the first 5 and last 10 minutes of the no drug period ($p<0.01$) (**Figure 44C**), contrary to what was observed in NCI baseline sessions when expression of maladaptive seeking patterns emerged. Lastly, as in the NCI baseline sessions, peak frequency did not differ between the first 5 and last ten minutes of the NCI no drug period, indicating that the expression of adaptive or maladaptive seeking patterns or the effect of the NCI is likely not associated with changes in peak frequency that are observable through fiber photometry (**Figure 44D**).

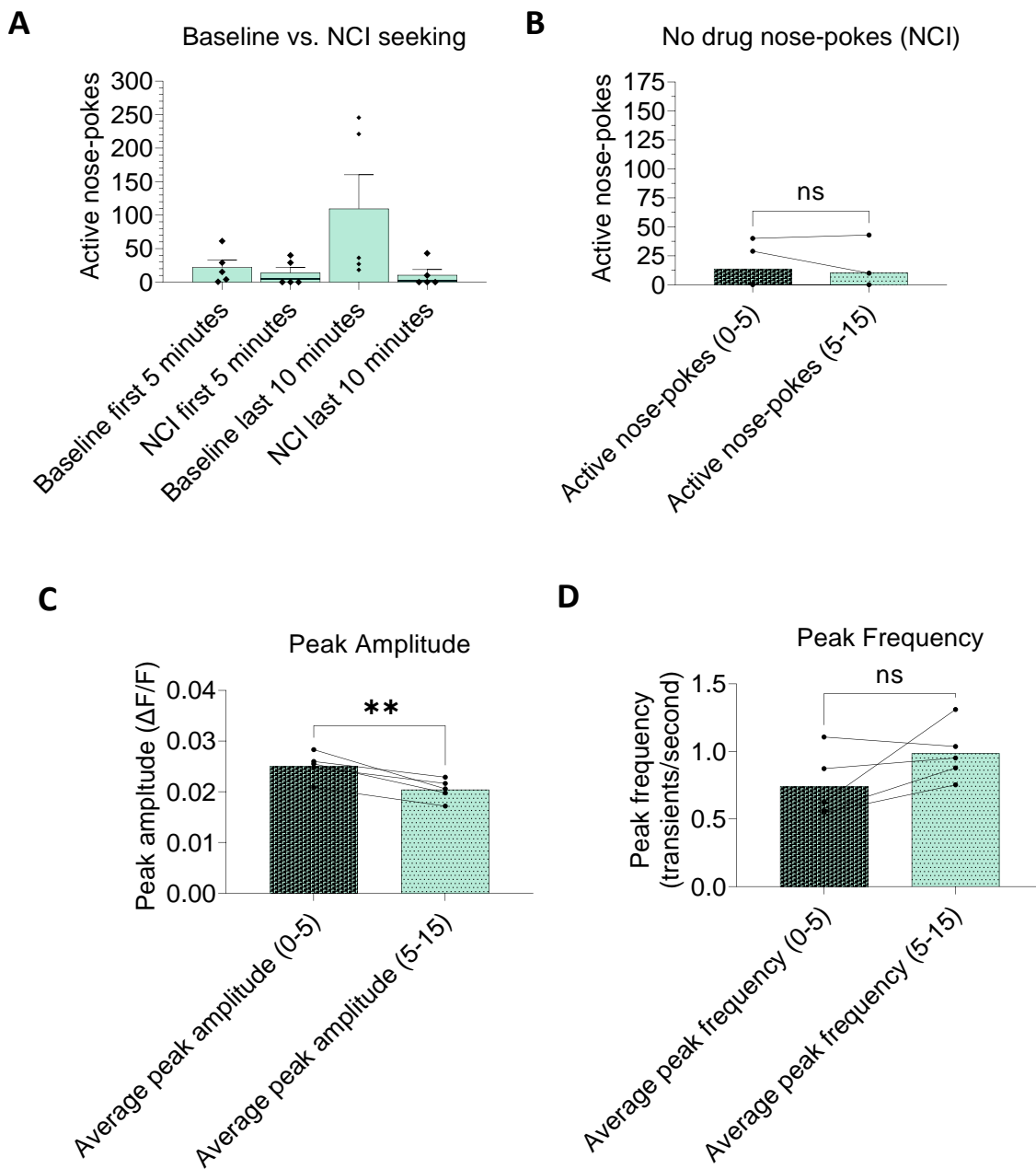


FIGURE 44: Effect of NCI on no drug seeking and PL activity – A. Though there was no effect of condition, time, nor condition x time, there was a visually-identified reduction in active no drug nose-pokes when comparing the average active nose-pokes in the last ten minutes of the previous no drug periods to the number of active nose-pokes in the last ten minutes of the NCI no drug period. B. Rats did not have significantly different behavior in the first 5 minutes compared to the last 10 minutes of the NCI no drug period. C. There was decrease in the peak amplitude from the first five minutes to the last ten minutes of the NCI no drug period. D. The peak frequency did not significantly differ between the first five minutes and last ten minutes of the NCI no drug period.

DISCUSSION

Fiber photometry recordings in the PL showed that expression of maladaptive seeking patterns was associated with a trend indicating an increase in the amplitude of peaks. Accordingly, non-contingent injections of cocaine (that prevent maladaptive seeking patterns from emerging) are associated with a decrease in the amplitude of peaks. However, these changes are not associated with changes in peak frequency. This is not uncommon, as other studies have evidenced that changes in amplitude, but not frequency, are observed in fiber photometry studies (Muir et al., 2018). While the dissection of the interpretation of differences in amplitude but not frequency is complicated, an increase of amplitude and not frequency is likely due to increased synchronicity of firing from neurons or simply higher firing in an increased number of neurons. However, as fiber photometry is a bulk fluorescence tool and has limitations in temporal resolution, a precise interpretation of this change cannot be concluded and needs to be explored further.

As evidenced in chapter one, globally, rats did have significantly lower seeking in a no drug period in a drug free state (no drug pre session) compared to a no drug period under drug effect. Also as evidenced in chapter one, when split in high and low depending on their seeking patterns in intoxicated no drug period, individuals expressing maladaptive seeking patterns while intoxicated are still able to decrease this maladaptive behavior in a no drug period in which they are not under drug effect. The data was replicated here in a group of animals that underwent fiber photometry recordings during sessions initiated by a no drug period.

Despite there being a difference in behavior between no drug pre period and no drug period under drug effect, the fiber photometry recordings performed on these sessions showed that the recruitment of the PL was identical in both conditions at the population level. However, when split in high and low depending on their seeking patterns during the intoxicated ND period of the recorded sessions, there was a trend that high rats had a decreased recruitment of the PL in response to the inhibitory DS when intoxicated compared to in a drug-free state. Oppositely, low rats seemed to have an increased recruitment of the PL in response to the no drug DS when intoxicated compared to drug free state.

Through the fiber photometry recordings, it was evidenced that the extent in which the PL is recruited has a relationship with maladaptive seeking expression. Though these results are correlational and a causal role still needs to be explored, the data suggests PL recruitment in response to an inhibitory DS while intoxicated is necessary to prevent maladaptive seeking patterns. This correlation data in combination with the trend found in high/low rats in the

intoxicated no drug period, strongly suggests that in vulnerable animals, recruitment of the PL in response to the inhibitory DS does not occur when the animal is intoxicated, whereas in resistant (low) rats, the ability to recruit the PL in response to an inhibitory discriminative stimulus is intact, and as a consequence, the discriminative can be properly used to guide appropriate behavior.

Not only does the PL seem to have an important contribution to the integration of discriminative stimuli in guiding behavior, but the previous relationship between the peak amplitude and expression of maladaptive seeking behavior suggests that PL firing changes are associated with changes in maladaptive seeking expression, suggesting that PL activity changes are strongly associated with a key behavioral feature of problematic cocaine use: maladaptive drug seeking.

In resistant animals, the external discriminative stimulus (no drug DS) is sufficient to cause PL activation and consequently, can be integrated to guide appropriate seeking. However, in the vulnerable (high) rats, the event that recruits the PL and is used as the discriminative to guide behavior is rather a change in cocaine concentration. This shows that the PL is still responding to cues to guide appropriate behavior, however the discriminative stimulus used to guide behavior in the low rats seems to be the no drug light, while cocaine is the discriminative stimulus in the high rats.

The extent of recruitment of the PL is not shown to be related to previous intake. While the relationship between the PL recruitment and the number of injections and intake rate in the previous drug period are both approaching significance, the latency to the last injection is strongly not correlated, showing that the pharmacological effects of cocaine are likely not the cause of the individual differences shown in recruitment of the PL as a function of maladaptive seeking. The recruitment of the PL in response to the ND DS is concluded to be independent of intoxication level.

The fiber photometry recordings, while informative, still have considerable limitations. Particularly, fiber photometry recordings were performed with a virus that was specific for excitatory glutamatergic cells in the PL. As this virus lacks pathway specificity, the recorded neurons could be projecting to multiple different brain regions, making the resultant signal indistinct.

Future experimentation should focus on pathway specific fiber photometry recordings during self-administration using either a retrograde fluorescent indicator or by using a dual virus

approach. Through establishment of pathway specific fluorescent imaging, the activity of PL neurons during cocaine self-administration and consequently, maladaptive cocaine seeking, can be dissected as a function of addiction vulnerability.

Particularly, fiber photometry recordings occurring in the PL to NacC pathway are thought to be important in the incentive motivational value of cocaine in vulnerable compared to resilient populations. As this pathway has been shown to be crucial in individual vulnerability to food addiction as well as cocaine-specific reinstatement and craving behaviors, there is substantial evidence that the activity of this pathway may differ as a function of addiction vulnerability (Domingo-Rodriguez et al., 2020; James et al., 2018; Moschak & Carelli, 2021b; Siemsen et al., 2022).

Additionally, future experimentation can use optogenetics to inhibit the PL during the presentation of the ND DS to see the effect on maladaptive seeking expression. As recruitment of the PL at the moment of the initiation of the no drug period is correlated with adaptive seeking behaviors during the no drug period, it is hypothesized that an inhibition at this moment will prevent proper integration of the signal and result in a higher level of maladaptive seeking expression. From here, pathway specific modulation of PL pathways would give causal evidence for the role of these pathways in maladaptive seeking patterns and which pathways are significantly disrupted in individuals with problematic cocaine use.

CONCLUSION AND PERSPECTIVES

CONCLUSION

Cocaine use disorder has no approved pharmacotherapies and the current psychosocial therapies only have limited efficacy. As CUD only occurs in approximately 20% of cocaine users, there is also a need to understand what renders some users to develop problematic cocaine use while others can maintain controlled intake. These factors contribute to an increased need to understand the mechanisms that occur throughout the transition to addiction in the subpopulation of users that develop problematic cocaine use.

Notably, cocaine use disorders are highly associated with behavioral impulsivity phenotypes, including defaults in behavioral inhibition (Ahn et al., 2016). This is not quite a surprise, as individuals who have CUD frequently express maladaptive drug seeking behaviors. However, it is not known how much of this maladaptive seeking behavior is due to increased incentive motivational effects or defaults in behavioral inhibition, as both would promote drug seeking.

Through the use of a preclinical model of CUD, both individual variations and observations of maladaptive seeking patterns can be observed and the factors that influence the balance between incentive motivation and behavioral inhibition could be dissected. Importantly, the maladaptive seeking patterns that can be observed in some rats are reliably predictive of if the animal will transition to an addicted-like state.

The influences that seem crucial to the expression of this early maladaptive seeking, as shown in chapter one, seem to be simply due to the influence of cocaine. Contrary to the resistant rats, the vulnerable rats that express maladaptive seeking patterns during the signaled no drug periods seem to be highly sensitive to their internal state to guide behavior. Despite the environment signaling that drug is not available, the decrease in cocaine concentration (as a consequence of cocaine unavailability) acts as a stronger discriminative suggesting that re-initiation of cocaine seeking should occur. However, in an abstinent state, even the vulnerable rats can properly inhibit drug seeking behavior during a signaled no drug period, showing that without cocaine intoxication, the external environment is sufficient to prevent maladaptive seeking patterns. However, once cocaine is in the mix, it seems that the external discriminative stimulus loses its power for the vulnerable subpopulation.

The question was then if the two populations have differences in pharmacokinetics that could be responsible for this difference of impact of cocaine in the high and low groups. It was shown that the rate of intake in which the individuals normally self-administered had a tie with

pharmacokinetic differences. Lower cocaine concentration in the PL after an equal dosing regimen was found in animals that normally self-administered the drug at a faster rate, whereas the slower takers had higher levels of cocaine in the brain. Variations in cocaine distribution/PK could affect the pattern of use, for example, promote a fast pattern of use.

Through metabolite analysis, it was also shown that the production of EME from cocaine was different as a function of intake patterns: the faster/more cocaine taken normally, the higher proportion of the metabolite to cocaine, meaning a seemingly faster metabolism in the fast takers compared to the slow takers. As this pathway relies on BChE, plasma BChE activity was analyzed to see how it related to cocaine self-administration patterns. However, the faster the intake patterns in this case, the lower the BChE activity, which was contradictory to the previous results. However, this is most likely due to the fact that the animals were in withdrawal for the blood sampling while intoxicated for the brain concentration analysis.

Differences in cocaine distribution/metabolism were not directly related to the expression of maladaptive seeking. Interestingly, however, they could be related to the dynamic of maladaptive seeking over sessions. Although this must be explored more in depth, rats with low brain cocaine concentration after equal administration appear to show an increase in maladaptive seeking starting self-administration session 7.

Variations in cocaine distribution/PK promoting a fast pattern of use and resulting in potential differences in sensitivity to cocaine could promote a progressive loss of control of the external adaptive inhibitory DS over cocaine seeking, with the cocaine itself serving as the discriminative used to guide behavior.

Lastly, in animals expressing maladaptive seeking patterns, there was an observation of increased average PL peak amplitude during the period in which maladaptive seeking was expressed as well as weaker recruitment of the PL in response to presentation of the DS signaling that seeking behavior should be inhibited when in an intoxicated state. When drug free, maladaptive seeking patterns were not observed, and recruitment of the PL was no different from the controlling seekers. This signals that intoxication in vulnerable animals seems to lead to stronger disruptions in discriminative processing, where the internal state guides behavior more than environmental stimuli. This is correlated with a differential PL recruitment in response to a discriminative stimulus that should be integrated into guiding behavior; however, this difference seems to only be strongly observed when in an intoxicated state.

These studies highlight important factors that differentially affect vulnerable and resistant populations related to the capacity of behavioral inhibition. Resistant rats use the external discriminative to guide behavior even when intoxicated, whereas vulnerable rats use cocaine as a discriminative when intoxicated, which are associated with differences in PL functioning as a consequence of the differences of discriminative used to guide behavior.

Initially in vulnerable rats, as it is shown here, this default is reliant on the pharmacological effect of cocaine, however, after extended access, these defaults are chronic. However, uncovering the mechanisms of how cocaine differentially regulates synaptic plasticity in the prefrontal regions that are associated with adaptive inhibition still needs to be elucidated and could be crucial in finding effective therapeutics that focus on reversing the plasticity changes that occur as a result of cocaine use.

As cocaine use disorder results in prefrontal hypoactivity, it is possible that contingency management is efficacious due to continuous repetition of behavioral inhibition to meet the criteria for the non-drug reward (Connolly et al., 2012). However, the neurobiological correlates explaining the success of contingency management still need to be uncovered.

An additional prospective therapy could be done through transcranial magnetic stimulation or neurofeedback focused on the prefrontal region to rescue the drug-induced hypoactivity: restoring behavioral inhibition deficits that were caused by cocaine use. These methods have shown to be successful in both humans and rats, justifying their therapeutic potential (Corominas-Roso et al., 2020; Scarpino et al., 2019; West et al., 2021).

PERSPECTIVES

Additional analysis could be performed that look at the rate of intake as the defining criteria rather than high and low ND seeking. Despite the fact that the no drug seeking is the maladaptive behavior that emerges early in self-administration, looking at rates of intake could be equally informative: as it has been for the comparison of intermittent and extended access models in uncovering changes in cocaine-induced incentive motivation. Understanding if and how the rate of intake influences expression of maladaptive seeking could hold important information regarding the plasticity changes that occur in the transition to addiction as a result of pharmacokinetics.

There also is the potential for a more controlled experiment that also could be highly interesting and informative in which the pattern of intake is regulated (as done in Martín-García et al.,

2014). This could uncover how intake patterns influence maladaptive seeking and how this relates to disengagement of the PL in response to discriminative cues signaling that behavioral inhibition is the optimal choice.

Importantly, full replication of these experiments in females should be done: as only a selection of experiments in this dissertation was performed in both males and females. However, before these can be done and published, the predictability of the transition to addiction based on the no drug responding pattern needs to be performed in females, as the 0/3-crit model has only been performed in males. Unfortunately, the time and the work that it takes to complete a cohort using the 0/3-crit model was not possible in the timeframe of my PhD, therefore the choice to prioritize the use of males was taken.

Additional experimentation exploring BChE activity could be fruitful. Repeating the experiment with more rats would be able to uncover the relationship more significantly between self-administration behavior and enzymatic activity. Additional experimentation could also be done by comparing BChE activity after cocaine administration to levels in withdrawal to see how the activity of BChE changes as a function of intoxication or abstinence.

Regarding the difference in brain cocaine distribution, a potential collaboration with the lab of Xavier Declèves in Paris could give more insight on if the aforementioned active transport across the blood brain barrier is responsible for the differences in cocaine distribution as a consequence of the rate of intake. However, as the technique they use requires the rats to be alive and transported to Paris, there are significant limitations in the experimental design that needed to be rectified before a plan was proposed.

As the fiber photometry recordings were selectively performed in the global PL glutamatergic neurons, there was a lack of pathway specificity that needs to be done in the future. Trials were performed during the duration of the thesis to incorporate pathway specificity using a retrograde GCaMP (viral injection in the NacC, fiber implantation in the PL) but despite beautiful virus expression, there was no signal in the fiber photometry recordings. Future troubleshooting should be done to uncover the reason why signal was not obtained despite the neurons being healthy and having adequate viral expression. If the functionality of the retrograde virus is to blame, another approach with a dual virus approach (retrograde CAV2-Cre in the NacC and a Cre-dependent GCaMP in the PL) should be done (Bond et al., 2020; de Kloet et al., 2021). Fiber photometry recordings in neurons selectively projecting to the NacC can identify pathway-specific links between control of adaptive seeking behavior as a result of cocaine-induced incentive motivation in vulnerable and resistant populations.

Beside pathway specificity, specific cell types can be targeted to express the indicators depending on the promoter used in the viral construct, or through the utilization of transgenic mouse/rat lines (Luo et al., 2008), as commonly used for optogenetics and other viral constructs. Finally, fiber photometry can also be used in combination with electrophysiology and optogenetics in freely moving animals and recently has been used in combination with fMRI in head fixed animals (Liang et al., 2017; Patel et al., 2020). It is also possible to do dual color photometry with two different fluorophores- most popularly green (GCaMP6f) and red (jRGECO1a) (Meng et al., 2018). Altogether, this opens many perspectives for follow-up studies on the role of the PL in maladaptive cocaine seeking.

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