Identification of a genetic locus and environmental factors influencing initial cocaine sensitivity in C3H substrains

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INTRODUCTION

Cocaine use disorder (CUD) is highly prevalent and poses significant personal, economic and societal burdens. Despite the high prevalence, there are no FDA-approved treatments due to significant gaps in our knowledge about the etiology of CUD. The risk for developing a CUD is influenced by genetic background, the environment and complex gene by environment interactions.

Mice have been used successfully as an experimental system to identify genetic loci implicated in addiction-related behaviors. Previous studies have used crosses between genetically and phenotypically divergent inbred mouse strains resulting in the identification of genomic regions that span tens of megabases and contain hundreds to thousands of potentially causal SNPSs, hindering identification of the specific causal polymorphism. To overcome this issue, we are using a reduced complexity cross (RCC) between two closely related inbred mouse substrains.
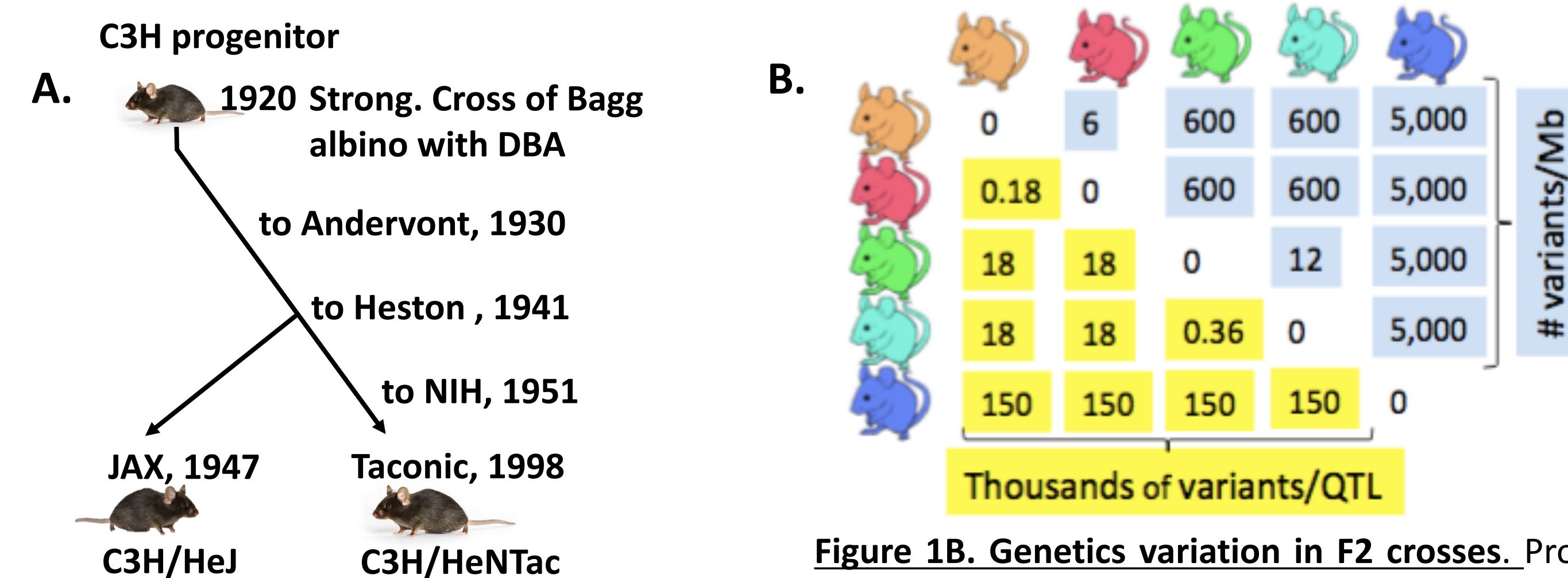

Figure 1A. Lineage relationship between selected C3H substrains.

Figure 1B. Genetics variation in F2 crosses. Provide a more simplified system for identifying and validating functional variants and their impact on complex traits (Image courtesy of FPM Villena).

Polymorphisms segregate between these subpopulations at regions of the genome for which the parental strain has not yet become fixed, or from genetic drift, resulting in sets of strains that are genetically very closely related. Inbred substrains harbor enough genetic polymorphisms to allow for genetic mapping but have a limited number of functional polymorphisms in mapped regions, thereby accelerating gene discovery. We identified a striking difference in cocaine-induced locomotor activation in HeJ and NTac substrains. An RCC detected one suggestive locus implying that non-genetic factors may also be contributing to the behavioral differences observed in the two substrains. Further investigation identified significant differences in the composition of the gut microbiota between these C3H substrains. This project aims to determine whether differences in the gut microbiota of these two substrains drive behavioral differences in sensitivity to the locomotor stimulating effects of cocaine. Establishing the role of the gut microbiome in the behavioral effects of cocaine provides a potential avenue for novel treatments.

## ACUTE COCAINE SENSITIVITY & BEHAVIORAL SENSITIZATION

Acute sensitivity to 5, 20 and 40 mg/kg cocaine was assessed using locomotor activity in the open field (OF). Behavioral sensitization was measured using a 19-day protocol during which mice received either an i.p. injection of saline (S) or cocaine (C).
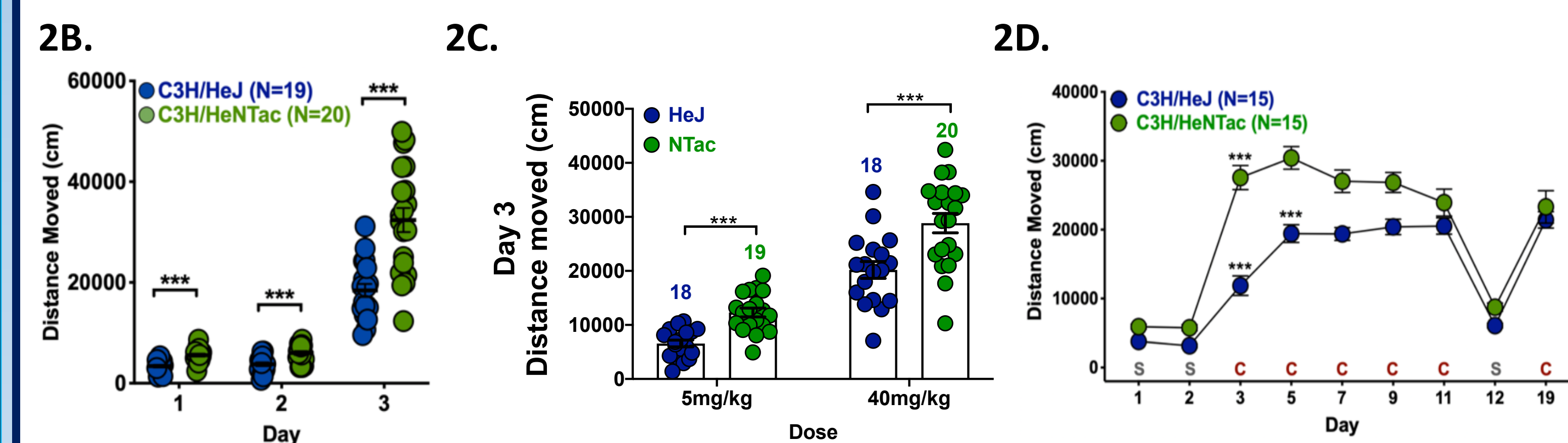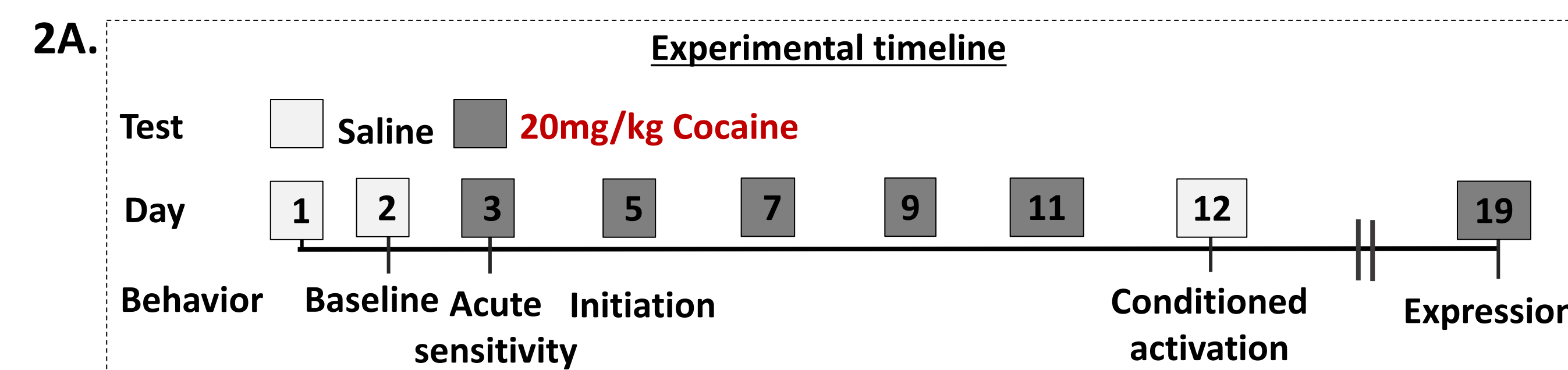
Figure 2. C3H substrains differ for acute cocaine sensitivity across multiples doses and for behavioral sensitization to cocaine. A. Experimental timeline. **B.** C3H/HeNTac (NTac) mice have significantly higher locomotor activation in response to 20mg/kg cocaine compared to C3H/HeJ(HeJ) mice ($p<0.001$), and **C.** at both lower (5mg/kg) and higher (40mg/kg) doses. **D.** HeJ mice displayed behavioral sensitization while NTac mice do not. For sensitization data, *** represent within substrain differences. All error bars are SEM.

INTRASESSION HABITUATION & ACUTE COCAINE SENSITIVITY

Intrasession habituation and initial locomotor sensitivity to cocaine were assessed during a 6-hour test measuring distance moved in the OF
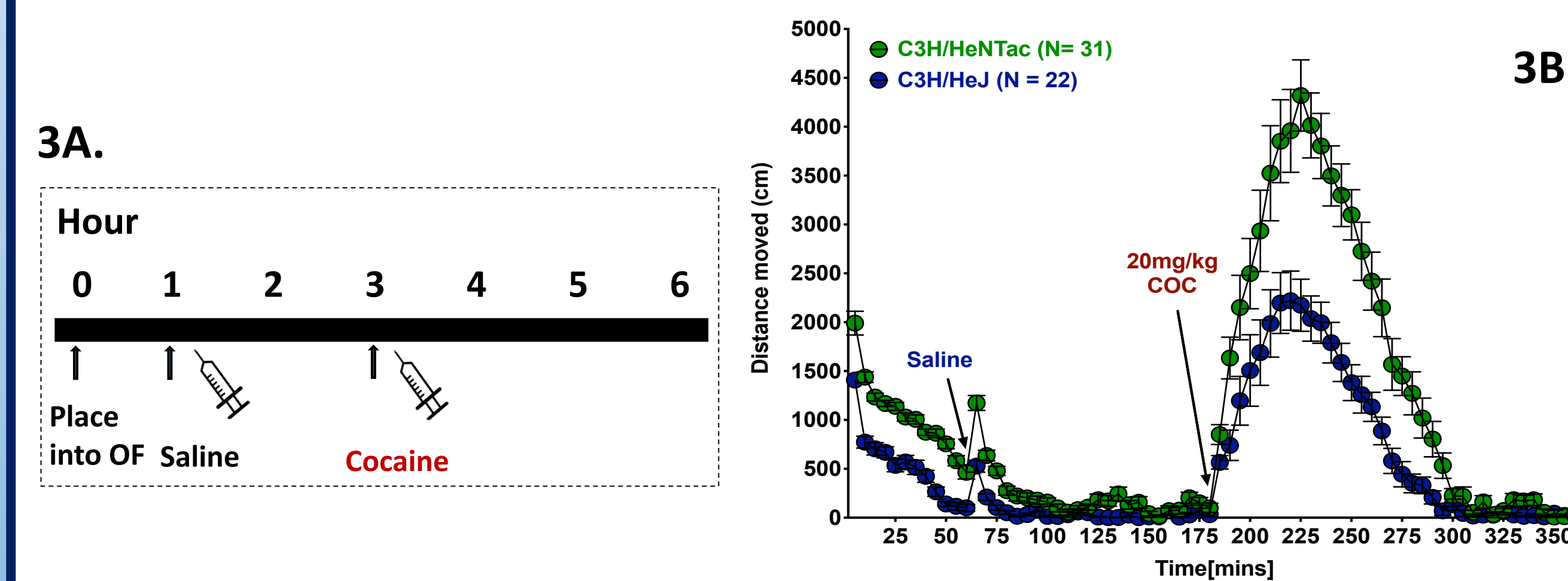
Figure 3. Acute locomotor difference in response to cocaine persists in C3H/HeNTac and C3H/HeJ following intrasession habituation. A. Experimental timeline. **B.** Both substrains show intrasession habituation and locomotor activity does not differ immediately prior to cocaine administration. C3H/HeNTac mice display higher initial locomotor activity and have a higher acute response to cocaine compared to C3H/HeJ mice. All error bars are SEM.

COCAINE PHARMACOKINETICS

Level of cocaine and its active metabolite norcocaine were measured in the brain using liquid chromatography.
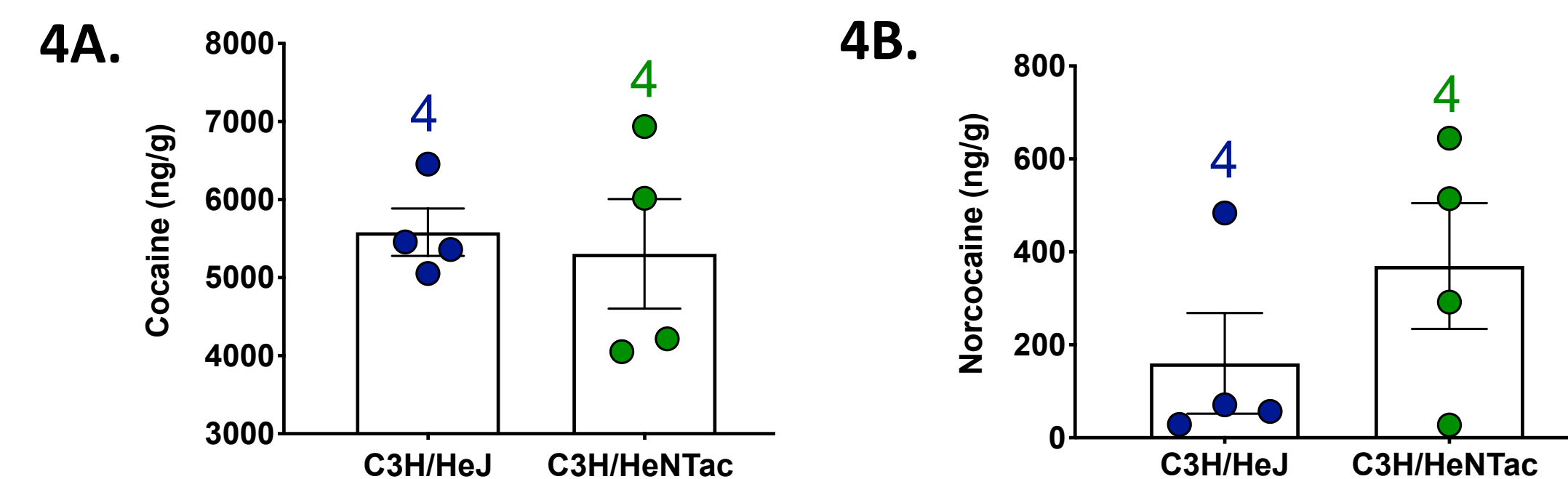
Figure 4. Substrains do not differ for cocaine or norcocaine levels in the brain. C3H/HeNTac and C3H/HeJ mice showed no difference in the amount of (**A.**) cocaine or (**B.**) its active metabolite norcocaine in the brain 15 minutes after an i.p injection of 20mg/kg cocaine. All error bars are SEM.

ACUTE COCAINE SENSITIVITY QTL MAPPING
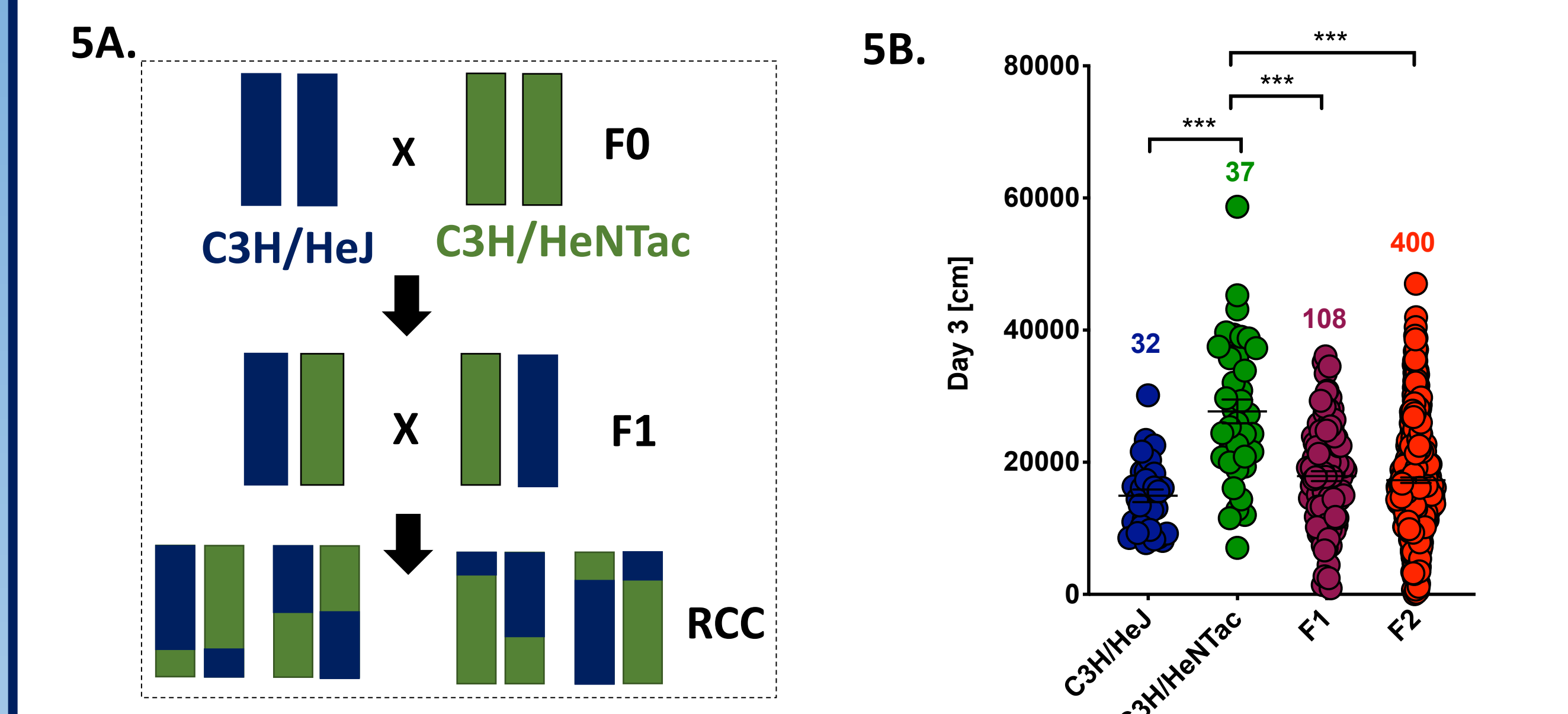
Figure 5. Low locomotor response is dominant in an F2/RCC population. A. C3H/HeNTac (NTac) and C3H/HeJ (HeJ) mice were used to make reciprocal crosses and a RCC mapping population. **B.** NTac show higher acute sensitivity to cocaine compared to HeJ mice. F1 and F2/RCC animals show acute sensitivity to cocaine similar to HeJ mice. All error bars are SEM.
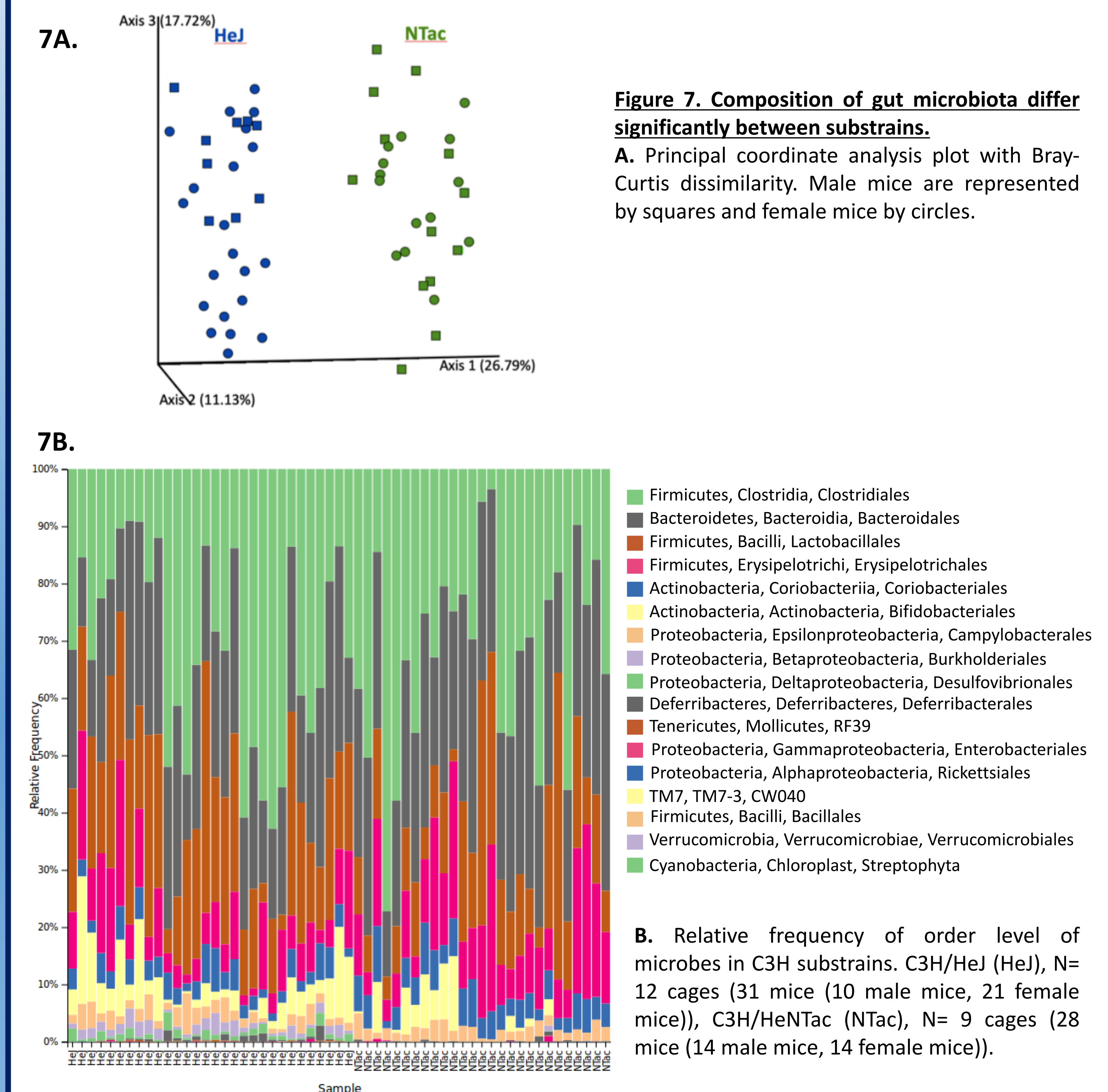
QTL ANALYSIS

MiniMUGA was used to genotype the RCC mapping population. R/qtl was used to identify genetic loci associated with the divergent phenotypic response.
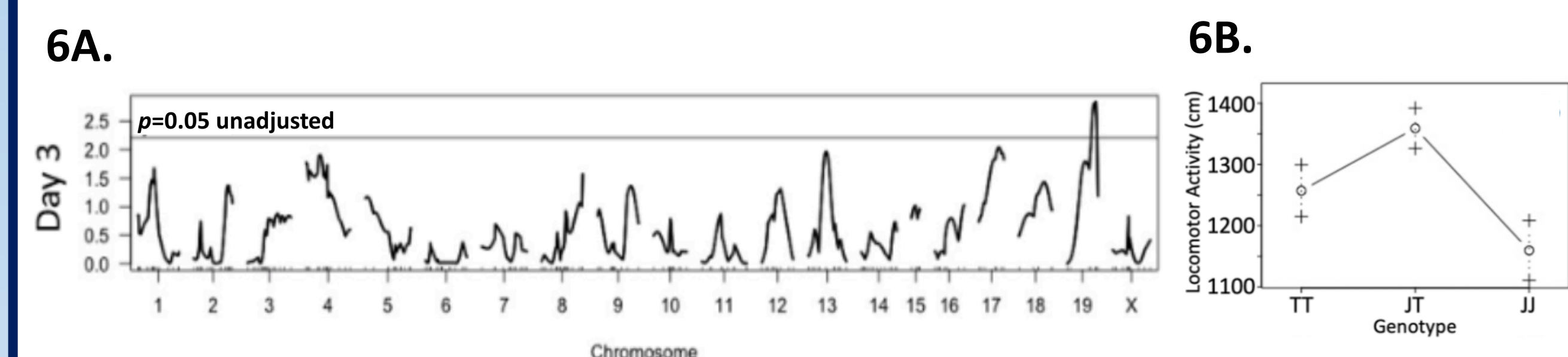
Figure 6. QTL analysis of cocaine-induced locomotor response (Day 3). A. A QTL was detected on Chr 19 (58Mb). **B.** NTac homozygotes (TT) and heterozygotes (JT) are more active than HeJ homozygotes (JJ).

COMPOSITION OF C3H SUBSTRAIN GUT MICROBIOTA

The gut microbial composition was assessed by Illumina MiSeq sequencing of the *16S rRNA* gene derived from fecal samples for each substrain.

Figure 7. Composition of gut microbiota differ significantly between substrains.
A. Principal coordinate analysis plot with Bray-Curtis dissimilarity. Male mice are represented by squares and female mice by circles.

B. Relative frequency of order level of microbes in C3H substrains. C3H/HeJ (HeJ), N= 12 cages (31 mice (10 male mice, 21 female mice)), C3H/HeNTac (NTac), N= 9 cages (28 mice (14 male mice, 14 female mice)).

CONCLUSIONS AND FUTURE STEPS

Conclusions:

- C3H/HeNTac mice have significantly higher acute sensitivity to 5, 20 and 40mg/kg cocaine compared to C3H/HeJ mice
- C3H/HeNTac mice do not show behavioral sensitization at 20mg/kg but this could be due to a ceiling effect at this dose. Lower doses should be tested
- Observed differences in cocaine-induced locomotor activation do not appear to be driven by a difference in levels of cocaine or its active metabolite in the brain. Additional doses, subjects and points of analysis should be tested
- A QTL on chromosome 19 was detected and the direction of the effect was as expected based on phenotype difference between the substrains
- The gut microbiota differs significantly between C3H/HeJ and C3H/HeNTac mice

Future Steps:

- Cross-fostering at birth will be performed to induce a permanent shift in the gut microbiota of nursing pups. Pups will be tested at ~60 days of age for acute cocaine-induced locomotor activity to determine if gut microbiota are responsible for differences in locomotor response to cocaine in these substrains
- Maternal care will be visually assessed twice a day from PND 2-5 to measure substrain-specific nurturing, non-nurturing and self maintenance behaviors
- Existing bioinformatic and behavioral data from additional C3H substrains has already been used to prioritize candidate polymorphisms under the Chr 19 QTL region for further examination

ACKNOWLEDGEMENTS