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Different Exercise Tendencies Modulate Behavioral and Molecular Changes to Opioid or Exercise-Induced Reward

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Different EXERCISE TENDENCIES modulate BEHAVIORAL and MOLECULAR CHANGES to OPIOID or EXERCISE-INDUCED REWARD

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Abstract

Reward changes were observed in rodents with different exercise tendencies by utilizing the conditioned place preference paradigm. Adult male Wistar rats with distinct phenotypes (low volume runners, high volume runners, and wild-type) were given access to a running wheel or an injection of morphine as a rewarding stimulus. There was no difference observed in the strength of conditioned place preference between the rewarding stimuli. Extinction was significantly more effective in low volume runners than high volume runners and wild-type animals, as was observed in the lower percentage of time spent in their assigned conditioning chamber. These findings suggest that low volume runners have a unique underlying genotypic difference, as well as a phenotypic difference from high volume and wild-type runners. It is also suggested that running wheel access and opioid administration are comparable rewarding stimuli in rodents.

Exercise Tendencies Modulate Changes to Reward

Physical activity is enjoyable for some adults, but others may have a difficult time initiating a workout. There is a widespread conviction that there are individual differences in the desire to exercise, which is somehow determined by phenotype. The "high" one experiences from aerobic exercise may be comparable to an opioid-induced high. Voluntary running appears to be rewarding for rats, and has been used as a stimulus in conditioned place preference (Raichlen, Foster, Gerdeman, Seillier, & Giuffrida, 2012). Access to a running wheel appears to be a motivating stimulus for rats that is comparable to the motivating factor of opioid administration (Brown, Green, Arthur, Booth, & Miller, 2015). There may be a difference in the strength of conditioning in CPP for animals with varying motivation to run. The current study looked at three distinct phenotypes for running motivation and the animals' tendency to be conditioned to a certain chamber in the CPP apparatus.

Hypotheses:

1. There will be a difference in the motivation to exercise among the various phenotype groups (LVR, HVR, WT).
2. Conditioned place preference will differ between drug and wheel access groups.
3. There will be a difference in conditioned place preference among the three distinct phenotypes : LVR, HVR, and WT.
4. A significant interaction effect will be observed among phenotypes and treatment groups.

Wheel running appears to be a rewarding experience for rodents and may act as a conditioning stimulus. The rewarding effects of voluntary wheel running outlast the duration of a running session. It was found that the aftereffects of running are rewarding rather than aversive and activates the reward system (Lett, Grant, Koh, & Smith, 2000). Greenwood et al. (2011) found that long term voluntary running has a rewarding effect that alters gene transcription in the mesolimbic reward pathway in particular. When rats are given more time to voluntarily run on the wheel, the rewarding effects linger for longer when they enter an inactive period (Sherwin, 1998). It is argued that the effects long term running has on the mesolimbic reward pathway will also change the rewarding component of drug abuse and help to cope with stress (Greenwood et al., 2011). Lett, Grant, and Koh (2001) also looked at how the rewarding effects of wheel running can be mediated by endogenous opioids. They found that naloxone attenuated the conditioned place preference induced by wheel running. It was concluded that endogenous opioids mediate the rewarding effects of running (Lett, Grant & Koh, 2001).

Brief or intermittent voluntary exercise on a running wheel may produce a cross-tolerance to opiates since running attenuates conditioned place preference to morphine (Eisenstein & Holmes, 2007). The rats that were in a running condition showed a stronger conditioned place preference to morphine by increased amount of time being spent in the morphine-paired chamber. Additionally, rats that ran on wheels voluntarily had less preference for sucrose water than rats that were sedentary (Eisenstein & Holmes, 2007).

Wheel running is a similar reward to morphine in conditioning. Lett, Grant, Koh, and Smith (2001) found that wheel running can be effective at conditioning taste aversion in rodents similar to morphine. This shows that morphine and running on a wheel are comparable rewarding stimuli for rodents. Milekic, Brown, Castellini, and Alberini (2006) found that an established morphine conditioned place preference may be disrupted by blocking protein synthesis after a conditioning session. With this finding, it has become apparent that drug conditioning can be disrupted in drug abusers (Zschucke, Heinz, & Ströhle, 2012). Conditioned place preference (CPP) is used to model drug addiction and relapse.

The molecular bases of changes during morphine-induced CPP include the activated MEK-ERK pathway in the ventral tegmental during drug reinstatement (Lin, Wang, Ji, & Yu, 2010). Mesolimbic dopamine (DA) pathway also plays a role in morphine addiction by increasing the drug's motivational effects. The DA, D_1, and D_2 receptors in the nucleus accumbens shell play a role in the acquisition of morphine-induced CPP (Fenu, Spina, Rivas, Longoni, & Chiara, 2006). Previous research has established that voluntary access to a running wheel is a rewarding stimulus for rats that is comparable to the rewarding effects of morphine. Both stimuli are effective at establishing a conditioned place preference. Individual differences in the animals' tendencies to run need further investigation to gain a better understanding of the underlying mechanisms.

Method

Animals

The animals used were adult male Wistar rats. They were kept at regulated ambient temperatures of 20℃ - 22℃. Inverted light cycles have shown to improve the aerobic condition of male Wistar rats (Manchado-Gobatto et al., 2008). In order to avoid artificially inflating the animals' running activity, they remained on a regular 12 hour light/dark cycle (lights on at 07:00). They were housed in polyethylene cages (29.2 x 19.1 x 12.7 cm). The animals had unrestricted access to water and food at all times. The Wistars used had three distinct and reliable phenotypes: high-volume runners (HVR), low-volume runners (LVR), and wild-type (WT). The animals were selectively bred at UM-Columbia. A total of 76 animals were used.

Apparatus

For the exercise treatment, the animals were given access to a stainless steel running wheel with a diameter of 34.5cm and width of 9.7cm. In addition, a conditioned place preference (CPP) apparatus constructed from Plexiglas was used. The paradigm was made up of two boxes with equal side compartments (60cm x 30cm x 30cm) and a small central start box (13cm x 13cm). The two sides of the apparatus were distinguished by the background and flooring. The background of the walls were either dotted or striped; the flooring was either a smooth painted wood surface or a textured grid. The CPP apparatus has shown to be effective in reinforcing rewarding pharmacological stimuli, such as addictive drugs. CPP is a learned behavior that rats may experience when a rewarding event is paired with a specific place. With this paradigm, it is possible

to measure drug reward in rats (Tzschentke, 2007). The animals' movements were tracked by an overhead camera using ANYmaze software. Between each trial, a solution of 70% ethanol was used to clean the apparatus which quickly evaporates.

Experimental Design

There are six distinct groups distributed in a 2 x 3 factorial design. The independent variable includes two treatments, exercise and drug groups. For the exercise treatment, the animals had 12 hour access to running wheels prior to behavioral testing. The drug group received morphine injections of 5 mg/kb/bwt instead of access to running wheels. Three distinct, reliable phenotypes were identified by colleagues at the University of Missouri at Columbia. These phenotypes include low volume runners (LVR), high volume runners (HVR), and wild-type (WT).

All animals used in behavioral testing had 7 days of free access to running wheels to express their phenotypic differences. Then, a baseline preference was completed to identify the rat's affinity for a specific chamber. The rats were randomly assigned to a conditioning chamber, where they received their assigned rewarding stimuli (either morphine injections or access to a running wheel 12 hours prior). Every other day, the drug group received saline injections. In contrast, the running treatment group received saline injections every day of conditioning. After 8 days of conditioning, the animals entered the extinction phase, where no rewarding stimuli was presented. At this stage of the experiment, they were given free access to both chambers of the CPP apparatus. After 8 days of extinction, the animals received a replacement of the initial motivation for reinstatement testing (Aguilar, Rodríguez-Arias, & Miñarro, 2008).

Procedure

Behavioral testing for conditioned place preference (CPP) began after the animals were given 7 days of free access to running wheels. On day 1, baseline preference was carried out for 30 minutes total. During this time, the animals had access to the entire apparatus. On days 2-9, the animals were conditioned to a randomly assigned chamber (either dotted + smooth floor or striped + textured floor). On alternating days of conditioning, the animals received a rewarding stimulus. Saline was administered on the "off" days in place of the rewarding stimulus. After either reward or saline injection, they had 30 minutes of access to one chamber. Place preference testing was carried out on day 10 where the animals receive a saline injection and access to both chambers. This was used to observe any preference the animal may have developed during conditioning. Days 11-18 consisted of the extinction phase, where no rewarding stimuli was presented. The animals were simply given access to the entire apparatus for 10 minutes each day. On day 19, the reward was given once more and the animals had access to both chambers. The animal's preference for either chamber was recorded. The animals were then sacrificed on day 20 after receiving their designated reward.

Results

In phase one, the distinct phenotypes were confirmed after running a one-way analysis of variance (ANOVA) on average running times and distances of individual animals. The data used to conduct this analysis were collected from the 7 days of voluntary running that took place prior to the start of behavioral testing. The average daily running times and distances of each animal were compared to see if there was a

significant difference in running times as predicted. A significant main effect was observed with running times, $F(2,53) = 26.16$ $p < .001$; $\eta^2 = 0.50$ [Figure 1], and distances $F(2,53) = 19.00$ $p < .001$; $\eta^2 = 0.42$ [Figure 2]. The partial eta squared values show that the main effects observed have a large effect size.

Following the one-way ANOVA, post hoc comparisons using Tukey's HSD test showed how the three phenotypes differed. In this analysis, it was found that LVR (M = 320.95, SD = 283.61) run times (seconds) were significantly lower than the HVR (M = 2834.00, SD = 1707.54) and WT (M = 2022.60, SD = 919.43) animals. In addition, the LVR (M = 0.15, SD = 0.144) animals ran significantly shorter distances (km) than the WT (M = 1.14, SD = 0.72) animals and the HVR (M = 1.83, SD = 1.32) animals. HVR animals ran significantly longer distances than WT animals, but HVR and WT animals did not differ significantly in run times.

In phase 2, the time each animal spent in their assigned conditioning chamber was converted into a percentage for all eleven stages of testing (baseline, place preference, extinction days 1 through 8, and reinstatement) [Table 1]. A multivariate analysis of variance (MANOVA) was conducted to examine the relationships among phenotypes, treatment groups, and the percentages of time spent in the conditioned chamber during each stage of behavioral testing. Missing data (<5% total) was replaced with mean averages for each dependent variable. No multivariate outliers were identified and Box's M test was not statistically significant, indicating that assumptions of homogeneity were met, $X^2(12) = 20.05$, $p=0.07$ [Figure 3]. There was a significant effect of phenotype on the dependent variate (Wilks's lambda = 0.69; $F(11,62) = 2.47$, $p < .05$) [Figure 4, 6].

There was no significant main effect observed in the treatment variable on the dependent variate ($F(11,62) = 1.73$, $p = 0.09$) [Table 2, Figure 5, 6].

A series of three planned contrasts were conducted to follow up on the significant main effect of phenotypes to better understand how they differed in their effects on the observed outcomes. The first contrast, examining whether HVR and WT animals differed, was not statistically significant (Wilks's lambda = 0.80; $F(11,61) = 1.40$, $p=0.20$). The second contrast, examining whether LVR and WT animals differed, was statistically significant (Wilks's lambda = 0.68; $F(11,61) = 2.55$, $p<0.05$). The third contrast, examining whether LVR and HVR animals differed, was also statistically significant (Wilks's lambda = 0.63; $F(11,61) = 3.23$, $p<0.01$).

The univariate effects for place preference, extinction day 8, and reinstatement were evaluated to observe if phenotypes differed significantly in percentage of time spent in their assigned conditioning chamber. The univariate effects for place preference ($F(2,71) = 3.09$, $p=.052$) and reinstatement ($F(2, 71) = 0.96$, $p=0.39$) were not significant. The univariate effects for extinction day 8 ($F(2,71) = 11.67$, $p<.001$) was statistically significant. Post hoc analyses were conducted to analyze how the phenotypes differed on extinction day 8. It was found that the LVR ($M = 28$, $SD = 17$) animals spent a significantly smaller percentage of time in the conditioned chamber than WT ($M = 42$, $SD = 12$) and HVR ($M = 50$, $SD = 19$) animals. WT and HVR animals did not significantly differ in the percentage of time spent in the conditioned chamber.

Discussion

The results from phase 1 partially support the first hypothesis. The results indicate that the LVRs may be genotypically unique. They exhibited significantly lower run times and distances than the HVRs and WT animals [Figure 1, 2]. However, the WT and HVR groups did not differ significantly in run times, showing that they may be more phenotypically similar than anticipated. As expected, the HVRs had the highest average run times and distances and the LVRs had the lowest average run times and distances. The LVR animals' voluntary running patterns may be indicative of a unique ability to reach euphoria in a shorter amount of time, or they are simply not as motivated to exercise on the running wheels (Roberts et al., 2014). Running is a comparable rewarding stimulus to morphine for all phenotypes, so even LVRs do not clearly "prefer" one rewarding stimulus over the other. In terms of analyzing these three distinct phenotypes further, future studies may assess the pace changes each animal exhibited throughout a running period. This would allow for more detailed insight into the unique running patterns of each phenotype.

The analyses carried out for phase 2 data showed that there was a significant difference among the varying phenotype groups in place preference behaviors. Similarly to the phase 1 results, phase 2 shows that the LVR animals were unique in their observed behaviors. LVRs differed significantly from HVR and WT animals in the percentage of time spent in the conditioned chambers throughout behavioral testing stages. Specifically, LVR spent a significantly smaller percentage of time in their assigned chambers on the last day of extinction than HVRs and WTs. This indicates that they

were able to more quickly extinguish the association between their conditioned chamber and the rewarding stimuli, regardless if the stimuli was drug or wheel access. WTs and HVRs were statistically similar in their observed behaviors within the conditioned place preference paradigm as expected. This further emphasizes the unique behaviors of the LVR animals and indicates that reinstatement has similar effects on all 3 phenotypes, absence of the rewarding stimuli over time allows for a much quicker extinction of the conditioned place preference.

There was no significant main effect observed for the treatment variable (drug and wheel-access). This indicates that the two rewarding stimuli were comparable [Table 2, Figure 6]. These findings support previous literature indicating that opioids are a comparable rewarding stimuli to voluntary running among rodents. Further analyses may be conducted to examine the possible genotypic differences of LVR animals. Additionally, further research may be conducted to observe other ways in which LVR animals exhibit unique behaviors.

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Table 1

Mean Percentage of Time Spent in Conditioned Chamber by Phenotype

	LVR		WT		HVR	
Measure	*M*	*SD*	*M*	*SD*	*M*	*SD*
Baseline	55%	33	57%	22	52%	23
Place Preference	41%	28	45%	17	57%	17
Extinction Day 1	41%	31	42%	19	45%	20
Extinction Day 2	36%	32	49%	23	39%	19
Extinction Day 3	40%	33	40%	19	47%	23
Extinction Day 4	39%	29	44%	27	39%	20
Extinction Day 5	47%	34	48%	21	50%	20
Extinction Day 6	32%	21	41%	15	49%	16
Extinction Day 7	31%	17	48%	19	49%	17
Extinction Day 8	28%	17	42%	12	50%	19
Reinstatement	43%	31	48%	20	54%	23

Table 2

Average Percentage of Time Spent in Conditioned Chamber Across IV Groups

	WT Runner		WT Drug		HVR Runner		HVR Drug		LVR Runner		LVR Drug	
Measure	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Baseline	53%	25	59%	25	39%	23	63%	22	35%	15	64%	34
Place Preference	48%	21	43%	17	51%	15	62%	16	36%	16	44%	33
Extinction 1	47%	15	39%	16	38%	21	51%	21	34%	22	44%	35
Extinction 2	51%	27	48%	14	33%	26	44%	22	39%	22	35%	35
Extinction 3	44%	22	38%	20	39%	28	55%	18	39%	23	40%	35
Extinction 4	55%	22	38%	10	40%	17	38%	28	41%	26	38%	34
Extinction 5	61%	21	41%	22	48%	23	53%	19	48%	19	46%	39
Extinction 6	46%	16	38%	14	42%	18	55%	14	40%	17	28%	22
Extinction 7	51%	14	47%	10	45%	13	54%	21	38%	20	27%	17
Extinction 8	41%	6	42%	2	38%	13	60%	14	35%	21	26%	18
Reinstatement	55%	20	44%	22	50%	21	57%	19	47%	24	42%	36

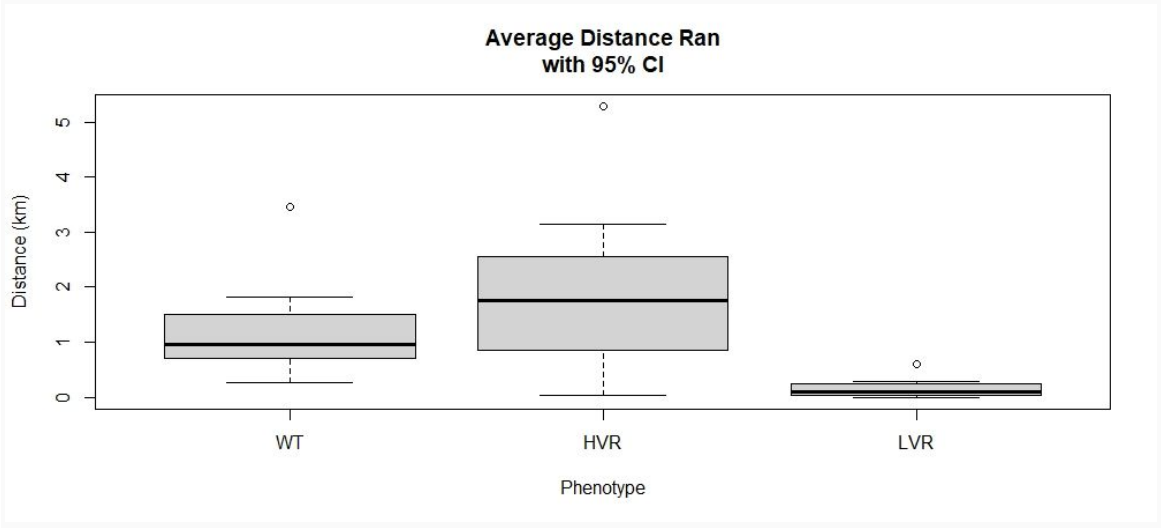

Figure 1. Box plot of average running distances across phenotypes

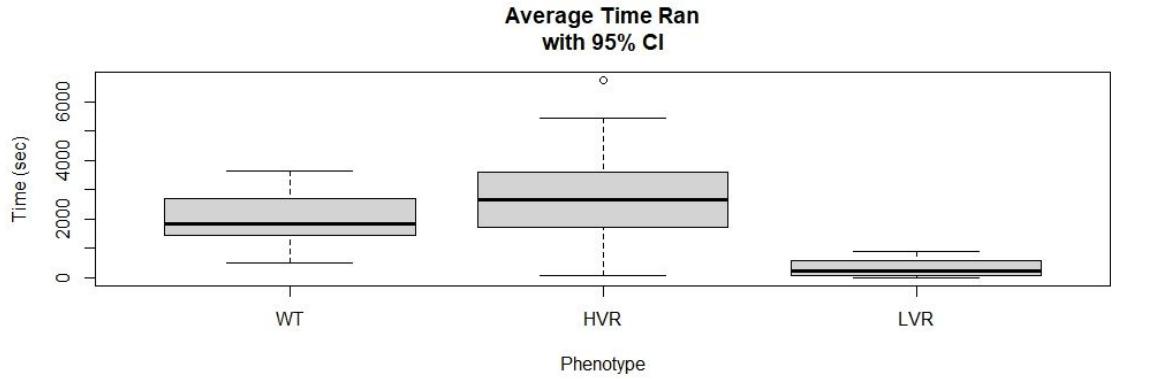

Figure 2. Box plot of average running times across phenotypes

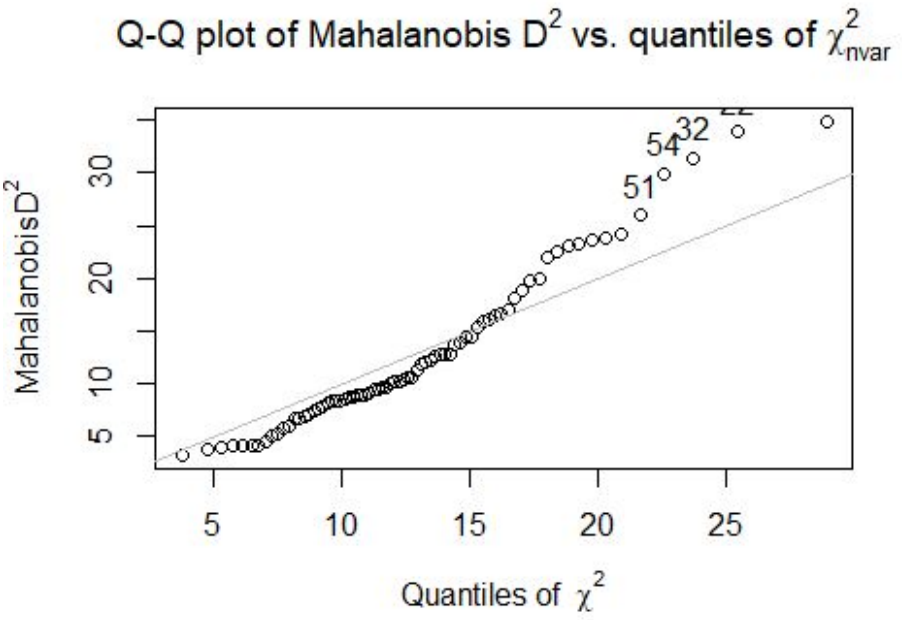

Figure 3. Phase 2 Homogeneity of Variance

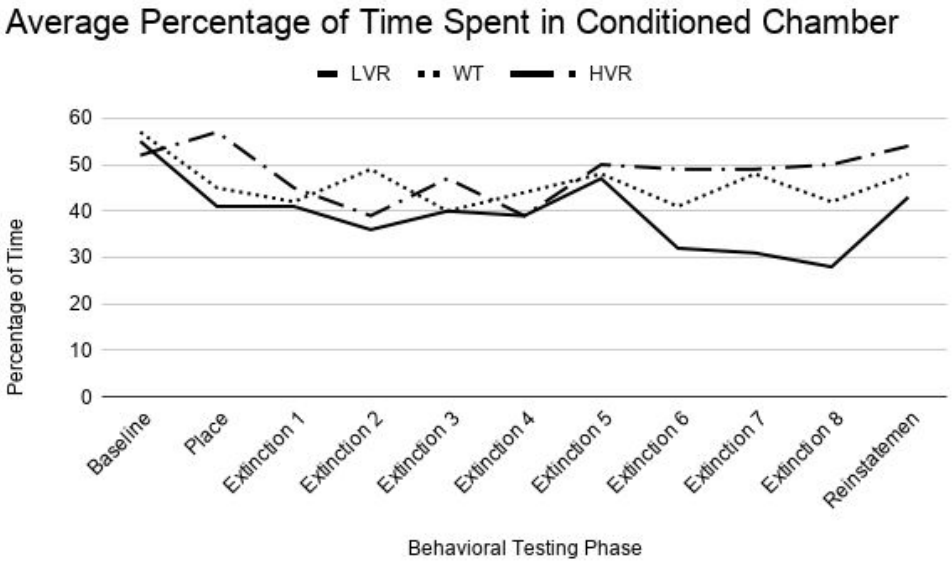

Figure 4. Average Percentage of Time Spent in Conditioned Chamber Across Phenotypes

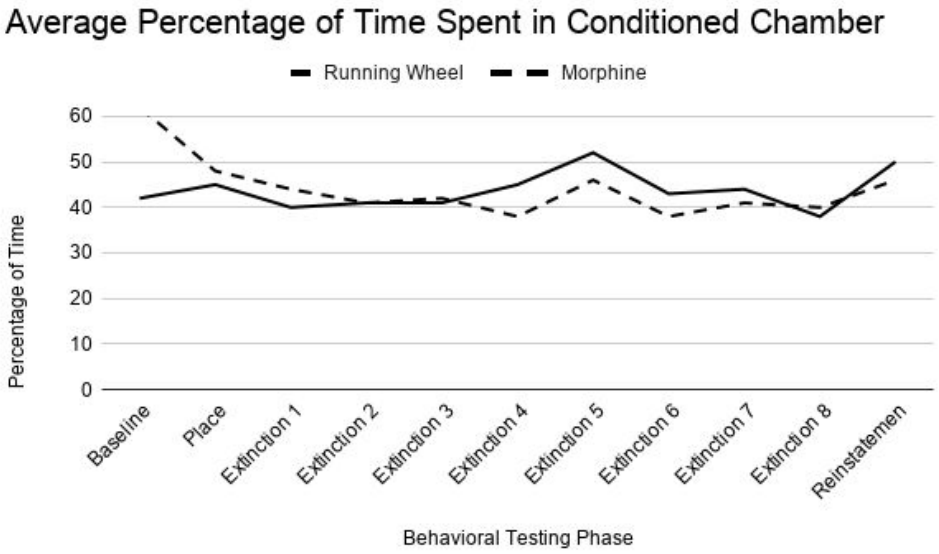

Figure 5. Average Percentage of Time Spent in Conditioned Chamber Across Treatment Groups

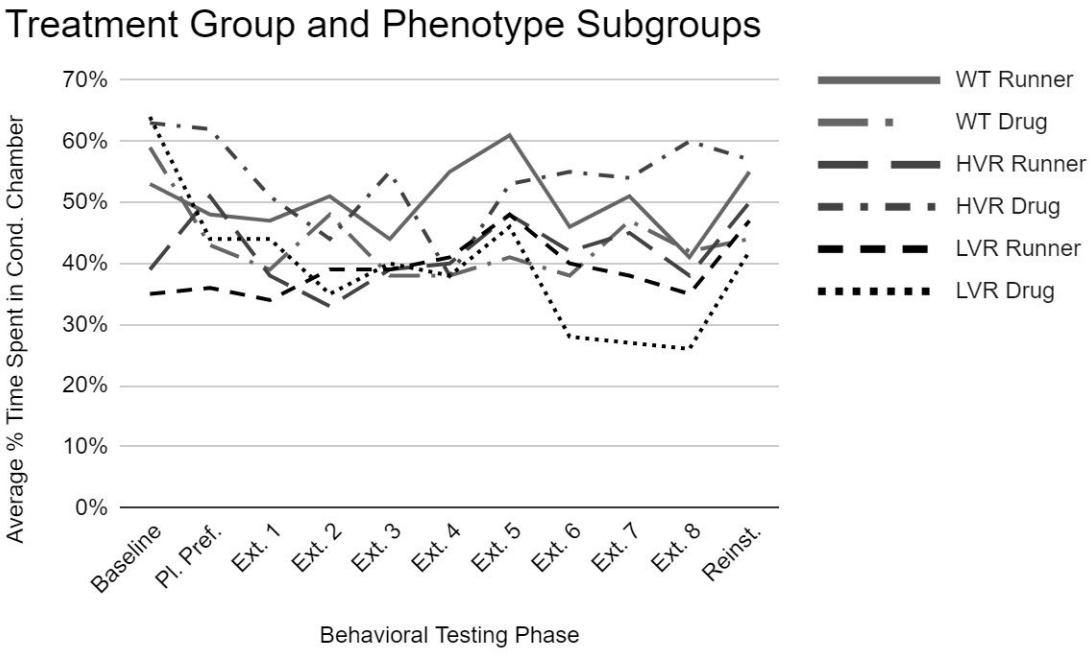

Figure 6. Average Percentage of Time Spent in Conditioned Chamber Across Independent Variable Groups