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Ryan Murray 20 April 2011

The Effects of CMS on Rats Selectively-bred for Behavior Related to Bipolar-like and Depression-like Symptoms

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Abstract

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By Ryan Murray

The chronic mild stress (CMS) paradigm often uses preference for sucrose as a measure of hypothesized anhedonia in rats. However, this measure is rife with unreliability, an issue that could be due to genetic differences between rats. This study tested this hypothesis by subjecting rats selectively bred for affective disorder-like behavior to CMS. Five lines of rats selectively-bred for behavior related to affective disorders were used: Hyperactive (HYPER), Swim-test Susceptible (SUS), Swim-test Resistant (RES), Swim Low-active (SwLo), and Swim High-active (SwHi) rats. The reactions of these selectively-bred lines to CMS were compared to the reactions of non-selectively bred (NS) rats which were used as controls. In addition, both female and male HYPER and NS rats were examined. Sucrose intake and preference for sucrose, as determined by the proportion of total fluid intake that was water intake, were measured and analyzed. Food intake and dark and light phase motor activity were also measured. During CMS, stressed HYPER rats, both females and males, SUS, RES, and SwHi rats showed lower preference for sucrose than did non-stressed rats of the same lines. In contrast, stressed female NS rats did not show a different preference for sucrose than non-stressed female NS rats, and stressed male NS rats tended to show higher preference for sucrose than non-stressed male NS rats. Stressed SwLo rats also did not show a different preference for sucrose than non-stressed SwLo rats. The effects on preference for sucrose could not be explained by a change in caloric intake as evidenced by patterns in food and water intake. Taken together, these results suggest that genetics can influence the outcome of CMS with respect to effects of stress on preference for sucrose and thus the known behavioral characteristics of a rat line should be taken into consideration when selecting animals for use in CMS experiments.

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Table of Contents

Figure 28 – 82

In the early 1980's Richard Katz developed the chronic unpredictable stress paradigm for use as an animal model of depression (Katz et al., 1981; Katz, 1982). In the chronic unpredictable stress procedure, rats are subjected to a series of severe stressors for an extended period of time (weeks to months) in a random order so as to prevent habituation. Such stressors included forced swim in cold (4°C) water, electrical foot shocks, tail pinching, long periods of food and water deprivation, and cage shaking (Katz et al., 1981). Rats subjected to chronic unpredictable stress were found to display decreased motor activity in an open field test as well as a reduced response to an activating stimulus (Katz et al., 1981). Perhaps even more interesting was the finding that rats exposed to the procedure also experienced a disruption in the intake of a palatable liquid solution and that this deficit could be prevented with administration of the tricyclic antidepressant imipramine (Katz, 1982).

In the late 1980's Paul Willner developed a modified version of Katz's chronic unpredictable stress procedure. Willner termed his version of the procedure as "chronic mild stress" ("CMS"), a name meant to highlight the use of milder stressors than those used in Katz's procedure, such as shorter periods of food deprivation, forced swim in room temperature water, overnight illumination, and soiled cage bedding, among others (Willner et al., 1987). The intent in reducing the severity of the stressors was to more closely model the stressors found in everyday life as well as heed ethical considerations (Willner et al., 1987; Willner, 2005). The main focus of CMS was on the reduction of intake of a palatable sucrose or saccharine solution which Willner believed to indicate the presence of anhedonia in rodents (Willner, 1997). However, other measures have also been used since such as intracranial self-stimulation (ICSS) and place preference tests

(see Willner, 2005 for a list of example studies). There is no standard CMS procedure and the types and schedules of stressors used vary considerably between studies (e.g. Pucilowski et al., 1993; Streklova et al., 2004; Valverde et al., 1997).

CMS has not been without controversy, however. One issue is that despite Willner's insistence that the observed effect of decreased sucrose intake is indicative of a loss of preference to sucrose, very few studies have actually shown what could be considered a true loss of preference. This is an important distinction because a decrease in sucrose intake with no corresponding change in water intake could very well be indicative of only a stress-induced loss in all fluid intake instead of an actual preference loss for palatable solutions. An unambiguous preference loss would require that total fluid intake remain the same and thus water intake increase in order to compensate for any decrease in sucrose intake. A cursory literature review revealed only two studies in which animals showed both a decrease in sucrose solution intake and a simultaneous increase in water intake (Pucilowski et al., 1993; Strekalova et al. 2004). All other studies read showed only a decrease in sucrose intake but little or no change in water intake.

Perhaps an even greater issue is that in the 23 years since Willner introduced CMS the sucrose measure has been rife with unreliability (Willner, 1997, see attached commentaries). While some laboratories are able to get an effect from CMS on palatable solution intake, other laboratories get no effect. Even inside the same laboratory CMS effects on palatable solution intake can be sporadic. Indeed, Willner himself even had trouble reproducing his own findings after moving laboratories and animal providers (Willner, 1997).

One explanation for the unreliability of the sucrose intake measure that has been

investigated (e.g. Bielajew et al., 2002; Griffiths et al., 1992; Nielsen et al., 2000) is that differences between rodent strains results in some strains being more susceptible to the effects of CMS than others (Willner, 1997). However, the findings from studies investigating this potential explanation have been just as sporadic as the sucrose measure itself, with some studies claiming that a certain rat line may be more susceptible to CMS than another, only for another study to be published claiming that the exact opposite is true. For example, a published protocol for CMS states that one should use Wistar or Lister rats but avoid using other rat lines such as Long-Evans or Sprague-Dawley "due to differences in reactivity to stressful stimuli" (Papp, 1998). However, one study did not observe any effect in Wistar rats either in ICSS or sucrose solution consumption although a sucrose effect was found in PVG hooded rats (Nielsen et al., 2000). Yet other studies have seen sucrose consumption decreases in PVG hooded, Wistar, and Lister rats (Willner et al., 1996). Other studies have also seen significant sucrose consumption effects in both Long-Evans (Valverde et al., 1997) and Sprague-Dawley rats (Duncko, Brtko, et al., 2001; Duncko, Kiss, et al., 2001). Yet in contrast another study found that only female Long-Evans and Sprague-Dawley rats decreased sucrose consumption when exposed to CMS, and no effect was found when preference was measured instead (Konkle et al., 2003). These illustrative examples of contradictory findings convey the significant variability observed between studies in the effect on sucrose intake and that even when rat strains are taken into account the variability still persists unfettered.

A related, but seemingly under-researched possibility, is that individual animals with particular characteristics may be more susceptible to the effects of CMS than others, just as some humans are more susceptible to stress and depression than others (Willner et

al., 1992, Griffiths et al., 1992, Pucilowski et al., 1993). If true, such a finding would mean that CMS would be best studied with sub-lines of rats that are susceptible to depression-like symptoms. Such an experiment has been performed with the Flinders Susceptible Line (FSL) and Flinders Resistant Line (FRL) of rats which found that after being exposed to CMS FSL rats decreased consumption of a palatable saccharin solution and increased consumption of water, thus indicating what could be considered a loss of preference for the palatable solution (Pucilowski et al., 1993).

In this study, several lines of rats selectively bred for behavior related to affective disorders were subjected to a CMS procedure in an attempt to elucidate whether these qualities made them more susceptible to the effects of CMS than non-selectively bred Sprague-Dawley (NS) rats. In particular, five selectively bred lines of rats derived from Sprague-Dawley rats were studied: Hyperactive (HYPER), Swim-test Susceptible (Susceptible or SUS), Swim-test Resistant (Resistant or RES), Swim Low-active (SwLo), and Swim High-active (SwHi).

HYPER animals are distinctive for the fact that they exhibit increased spontaneous nocturnal ambulatory activity compared to normal animals, as well as a period (2-7 days) of markedly increased nocturnal ambulatory activity after being exposed to a stressor when young (3-5 months old) but prolonged decreased nocturnal ambulatory activity after being exposed to a stressor when older (10-14 months old) (Weiss et al., 2008). Due to these characteristics, it is believed that HYPER animals may be a potential endogenous model of bipolar disorder in rats (Weiss et al., 2008). SUS rats show reduced activity in a swim test after being exposed to a stressor whereas RES rats are, as their name implies, resistant to this effect on swim test activity (Weiss et al.,

2008). Thus, SUS rats appear to be more susceptible to the effects of stress than NS rats whereas RES rats appear to be more resistant to the effects of stress than NS rats. Furthermore, SUS rats are a very good screen for antidepressant drugs as evidenced by the fact that these rats have responded to every class of antidepressant drug as well as electroconvulsive therapy while not responding to a number of psychoactive drugs that are known to produce false positive results (Weiss et al., 2008). The SwLo rats show little struggle and much floating in a swim test even when they have not previously been subjected to any stressors (Weiss et al., 2008). In contrast, SwHi rats show much struggle and little floating in a swim test (Weiss et al., 2008). SwLo rats also exhibit a strong positive response to activating antidepressants including tricyclics when chronically administered (i.e., for two weeks) which suggests that the SwLo rat may be an endogenous model of atypical depression (Weiss et al., 2008). The SwHi rats, in contrast, do not have any reaction to antidepressant treatment (Weiss et al., 2008). In all experiments, NS rats were used as controls.

Methods

Animals and Housing Conditions

Prior to the start of the study, all rats were group-housed with 2-3 animals per cage and kept on a 12:12-h light cycle. Lights went on at 0700 hours, off at 1900 hours, and colony temperature was maintained between 20-22°C. During the study rats were housed individually in cages with light sensors that recorded motor activity 24 hours per day. Stressed and non-stressed groups were housed in separate rooms. Temperature and light cycle were kept the same as before the start of the study. Both before and during the

study water and food were provided *ad libitum* except during the food deprivation stressor administered to stressed groups as detailed later in this section.

Experimental Design

Prior to the start of CMS rats were subjected to a seven day baseline period. All variables were measured daily during this time except for sucrose intake which was provided for only three consecutive days starting on the third day of baseline and ending on the fifth day of baseline. Following the baseline period rats were divided into two groups: Stressed and non-stressed. Each group was balanced on the following variables (in order from greatest to least priority): Sucrose intake, dark phase motor activity, light phase motor activity, food intake, and water intake. In some cases not all variables could be balanced for, although sucrose intake and dark phase motor activity were always balanced.

After the baseline period was over, rats in the stressed group were subjected to the CMS procedure which lasted 27 days. The CMS procedure used in this study consisted of eight different stressors (see table 1) randomly repeated three times each during the course of the experiment. In addition, there were three days during CMS in which rats were subjected to no stressors. The exception to the randomness of the CMS schedule was a three day sequence of restraint, foot shock, and no-stress (in that order) that was used in every experiment after the first one and which always occurred during each sucrose exposure period other than during “baseline” when, of course, no stressors occurred (see below).

A 2% sucrose solution was given to rats for three consecutive days three times during the experiment: In the middle of the baseline period (days 3-5), in the middle of

the CMS procedure (days 17-19), and on the last three days of the CMS procedure (days 32-34). During this time both sucrose and water were always freely available.

Food intake and water intake were recorded daily. Sucrose intake was also recorded daily during the exposure period. Motor activity was constantly recorded throughout the experiment and separated between dark-phase activity and light-phase activity when analyzed.

Experiments

Experiment 1. This experiment studied female HYPER rats. Rats used in the study included 41st generation female HYPER rats (n=12) and female non-selected rats (n=12). Rats were divided equally into stressed and non-stressed groups such that each group consisted of n=6 HYPER and n=6 non-selected rats. At the start of the experiment, the ages of all HYPER rats ranged from 4-6 months old and all non-selected rats were 5 months old. Groups in this experiment were balanced for sucrose solution intake, food intake, dark phase motor activity, and light phase motor activity.

Experiment 2. This experiment studied male SUS and male RES rats. Rats used in the study included 44th generation male SUS rats (n=12), 44th generation male RES rats (n=12), and male non-selected rats (n=12). Due to room constraints, groups were divided unequally into stressed and non-stressed groups such that there was a 2:1 ratio of stressed-to-unstressed animals. Thus the stressed group consisted of n=8 SUS rats, n=8 RES rats, and n=8 non-selected rats (for a total of n=24 rats). The non-stressed group consisted of n=4 SUS rats, n=4 RES rats, and n=4 non-selected rats (for a total of n=12 rats). At the start of the experiment all SUS and RES rats were aged 7 months old and

the ages for the non-selected rats ranged between 7-8 months. Groups in this experiment were balanced for sucrose intake and dark phase motor activity.

Experiment 3. This experiment studied male HYPER rats. Rats used in the study included 43rd generation male HYPER rats (n=12) and male non-selected rats (n=12). Rats were divided equally into stressed and non-stressed groups such that each group consisted of n=6 HYPER and n=6 non-selected rats. At the start of the experiment all HYPER rats were aged 6 months old and all non-selected rats were 7.5 months old. Groups in this experiment were balanced for sucrose intake, food intake, water intake, dark phase motor activity, and light phase motor activity.

Experiment 4. This experiment studied male SwHi and SwLo rats. Rats used in the study included 40th generation male SwHi rats (n=12), 40th generation male SwLo rats (n=12), and male non-selected rats. Due to room constraints, groups were divided unequally into stressed and non-stressed groups such that there was a 2:1 ratio of stressed-to-unstressed animals. Thus the stressed group consisted of n=8 SwHi rats, n=8 SwLo rats, and n=8 non-selected rats (for a total of n=24 rats). The non-stressed group consisted of n=4 SwHi rats, n=4 SwLo rats, and n=4 non-selected rats (for a total of n=12 rats). At the start of the experiment SwHi rats were aged three to three and a half months old, SwLo rats were aged three and a half months old, and non-selected rats were aged four and a half months old. Groups in this experiment were balanced for sucrose intake, food intake, water intake, dark phase motor activity, light phase motor activity, and body weight.

One SwLo rat from the stressed group died in the middle of the experiment and its data was subsequently removed from all statistical analyses.

Statistical Analysis

All variables of interest were analyzed using a three factor analysis of covariance (rat line X stress/no stress X day) with repeated measures across the day factor, and the covariate being the mean for that measure obtained from the baseline days on which the measure was made. In experiments with three rat lines, two rat lines were compared to each other using similar analyses in addition to the overall analysis that compared all three rat lines. Finally, stressed and non-stressed groups within the same rat line were compared using a two factor analysis of covariance (stress/no stress X day) with repeated measures across the day factor, with the covariate being obtained in the same manner as described above. In the analysis of both dark and light phase motor activity, data obtained on those days in which the overnight illumination and wet bedding stressors were administered were left out of the analyses (six days total) because these manipulations markedly decreased or increased ambulatory activity. Also, additional days were left out of the light phase motor activity analysis for each experiment if there were less than three hours of data available for that day due to the type and/or length of the stressor being administered on that day. In the analysis of light phase motor activity data, days were removed from the analysis if there were less than three uninterrupted hours of data available for that day due to the type and/or length of the stressor being administered on that day. As a result, for the light phase motor activity analysis Experiment 1 used 18 days in its analysis, Experiment 2 used 17 days, Experiment 3 used 24 days, and Experiment 4 used 18 days. In the analyses of both food and water intake, data obtained on those days on which the food deprivation stressor was administered (three days) were left out of the analyses since food intake was completely absent and

water intake was much lower than usual as a consequence on such days. In addition to the removal of the three food deprivation days, another six days on which rats were exposed to sucrose were left out of the analyses because the availability of sucrose resulted in decreased food and water intake. Thus, a total of nine days were left out of the food and water analyses.

Sucrose intake was analyzed in two different ways. One analysis included all days on which sucrose was available. As previously described, a three factor, repeated measures analysis of covariance was done, using mean sucrose intake during baseline (days 3-5) as the covariate for each rat. A second analysis was done which included only the first day of each sucrose exposure period. Again, data were analyzed using a three factor repeated measures analysis of covariance, using the first day of sucrose intake during baseline (day 3) as the covariate. The reason for considering only the first day of each sucrose exposure period is that it is a time period more often used in other CMS literature than the three day period also used in this study. While many CMS studies also use a 1-2 hour sucrose exposure period, such a short period has been reported to be not as accurate as a 24 hour exposure period (Ayensu et al., 1995; Hagan and Hatcher, 1997; Konkle et al., 2003).

Sucrose preference was also analyzed. The measure of sucrose preference was obtained by dividing the water intake by the total fluid intake for each rat on each day in order to obtain a ratio of water-intake-to-total-fluid-intake (WI:TFI). The higher this value was the less sucrose was preferred. Sucrose preference was then analyzed in the same fashion as sucrose intake as described above.

A small number of aberrant animals were not included in the sucrose or water analyses. These were animals that exhibited a sucrose preference of less than 50% on any day during baseline (in contrast, most rats exhibit a sucrose preference of 90% or greater); there were three such animals. In addition, one SwLo rat died during the experiment and was excluded from all analyses.

All experimental methods in the study were approved by the Emory University Institutional Animal Care and Use Committee (IACUC #214-2009). Pain and discomfort experienced by animals in during CMS was minimal, and any animals which experienced a decrease in body weight of 25% or more were removed from the study and euthanized.

Results

Sucrose Intake

HYPER rats. Figure 1 shows the sucrose intake of both stressed and non-stressed female HYPER and female NS rats. When all days in which rats were offered sucrose were analyzed by a three factor repeated measures analysis of covariance as described in the methods section, a significant difference between rat lines was found, $F(1, 18) = 12.958$, $p = .002$, which indicated that female HYPER rats showed significantly lower sucrose intake from that of female NS rats during the CMS phase. A significant interaction of rat line X stress/no stress was found, $F(1, 18) = 7.590$, $p = .013$, which indicated that female HYPER rats' sucrose intake was affected differently by stress from that of female NS rats. This result was supported by the evidence that stressed female HYPER rats showed significantly lower sucrose intake overall from that of non-stressed female HYPER rats, $F(1,9) = 12.184$, $p = .007$, whereas stressed and non-stressed female NS rats did not show significantly different sucrose intake, $F(1,8) = 2.428$, $p = .158$.

When only the first day of each exposure period was analyzed in a similar fashion, the significant difference between rat lines remained, $F(1, 18) = 6.909$, $p = .017$, while the interaction of rat line X stress/no stress was no longer significant, $F(1, 18) = 2.681$, $p = .119$. However, stressed female HYPER rats showed significantly lower sucrose intake overall from that of non-stressed female HYPER rats, $F(1,9) = 7.383$, $p = .024$, whereas stressed and non-stressed female NS rats did not show significantly different sucrose intake, $F(1,8) = 0.394$, $p = .548$.

Figure 2 shows the sucrose intake of both stressed and non-stressed male HYPER and male NS rats. When all days in which rats were offered sucrose were analyzed by a three factor repeated measures analysis of covariance as described in the methods section, a significant difference between rat lines was found, $F(1, 19) = 13.229$, $p = .002$, which indicated that male HYPER rats showed significantly lower sucrose intake from that of male NS rats during the CMS phase. The interaction of rat line X stress/no stress was significant, $F(1,19) = 4.625$, $p = .045$, which indicated that male HYPER rats' sucrose intake was affected differently by stress from that of male NS rats. This result was supported by the evidence that stressed male HYPER rats tended to show lower sucrose intake overall from that of non-stressed male HYPER rats, $F(1,9) = 3.916$, $p = .079$, whereas stressed and non-stressed male NS rats did not show significantly different sucrose intake, $F(1,9) = 1.147$, $p = .312$.

When only the first day of each exposure period was analyzed in a similar fashion, the significant difference between rat lines remained, $F(1, 19) = 20.015$, $p = .000$, and a significant interaction of rat line X stress/no stress was found, $F(1, 19) = 8.127$, $p = .010$. This result was supported by the evidence that stressed male HYPER

rats showed significantly lower sucrose intake overall from that of non-stressed male HYPER rats, $F(1,9) = 5.236$, $p = .048$, whereas stressed male NS rats tended to show higher sucrose intake overall from that of non-stressed male NS rats, $F(1,9) = 3.528$, $p = .093$.

SUS and RES rats. Figure 3 shows the sucrose intake of both stressed and non-stressed male SUS, male RES, and male NS rats. When all days in which rats were offered sucrose were analyzed by a three factor repeated measures analysis of covariance as described in the methods section, a significant difference between rat lines was found, $F(2, 27) = 11.637$, $p = .000$, which indicated that different rat lines showed significantly different sucrose intake from each other during the CMS phase. Further analyses comparing pairs of rat lines revealed that SUS and NS rats did not show significantly different sucrose intake, $F(1, 17) = 1.969$, $p = .179$, RES rats showed significantly lower sucrose intake from that of NS rats, $F(1, 19) = 9.389$, $p = .006$, and SUS rats showed significantly higher sucrose intake from that of RES rats, $F(1, 17) = 33.364$, $p = .000$. The effect for the interaction of rat line X stress/no stress was not significant, $F(2, 27) = .322$, $p = .728$, which indicated that stress did not affect different rat lines differently. This result was supported by the evidence that stressed and non-stressed SUS rats did not show significantly different sucrose intake, $F(1,7) = .000$, $p = .998$, stressed and non-stressed RES rats did not show significantly different sucrose intake, $F(1,9) = .211$, $p = .657$, and stressed and non-stressed NS rats did not show significantly different sucrose intake, $F(1,9) = .656$, $p = .439$.

When only the first day of each exposure period was analyzed in a similar fashion, the significant difference between rat lines remained, $F(2, 27) = 18.715$, $p =$

.000, and the interaction of rat line X stress/no stress was still not significant, F(2, 27) = .593, p = .560. Further analyses comparing pairs of rat lines revealed that SUS rats showed significantly higher sucrose intake from that of NS rats, F(1, 17) = 5.152, p = .037, RES rats showed significantly lower sucrose intake from that of NS rats, F(1,19) = 14.804, p = .001, and SUS rats showed significantly higher sucrose intake from that of RES rats, F(1, 17) = 52.272, p = .000. Stressed and non-stressed SUS rats did not show significantly different sucrose intake, F(1, 7) = .087, p = .776, stressed RES rats tended to show lower sucrose intake overall from that of non-stressed RES rats, F(1,9) = 4.639, p = .060, and stressed NS rats tended to show lower sucrose intake overall from that of non-stressed NS rats, F(1,9) = 3.718, p = .086.

SwHi and SwLo rats. Figure 4 shows the sucrose intake of both stressed and non-stressed male SwHi, male SwLo, and male NS rats. When all days in which rats were offered sucrose were analyzed by a three factor repeated measures analysis of covariance as described in the methods section, a significant interaction of rat line X stress/no stress was found, F(2,28) = 3.649, p = .039, which indicated that different rat lines' sucrose intake was affected differently by stress. Further analyses comparing pairs of rat lines revealed a significant interaction of rat line X stress/no stress between SwHi and NS rats, F(1, 19) = 6.221, p = .022, which indicated that SwHi rats' sucrose intake was affected differently by stress from that of NS rats. The interaction of rat line X stress/no stress between SwLo and NS rats was not significant, F(1, 18) = .634, p = .436, which indicated that stress did not affect SwLo and NS rats differently. The interaction of rat line X stress/no stress between SwHi and SwLo rats approached significance, F(1, 18) = 3.564, p = .075, which indicated that SwHi rats' sucrose intake tended to be affected differently

by stress from that of SwLo rats. These results were supported by the evidence that stressed SwHi rats showed significantly lower sucrose intake overall from that of non-stressed SwHi rats, $F(1,9) = 6.161$, $p = .035$, stressed and non-stressed SwLo rats did not show significantly different sucrose intake, $F(1, 8) = .152$, $p = .707$, and stressed and non-stressed NS rats did not show significantly different sucrose intake, $F(1, 9) = .828$, $p = .387$.

When only the first day of each exposure period was analyzed in a similar fashion the interaction of rat line X stress/no stress remained, $F(2, 28) = 3.518$, $p = .043$. Further analyses comparing pairs of rat lines revealed a significant interaction of rat line X stress/no stress between SwHi and NS rats, $F(1, 19) = 5.526$, $p = .030$, which indicated that SwHi rats' sucrose intake was affected differently by stress from that of NS rats. The interaction of rat line X stress/no stress between SwLo and NS rats was not significant, $F(1, 18) = .210$, $p = .652$, which indicated that stress did not affect SwLo and NS rats differently. A significant interaction of rat line X stress/no stress between SwHi and SwLo rats was found, $F(1, 18) = 4.719$, $p = .043$, which indicated that SwHi rats' sucrose intake was affected differently by stress from that of SwLo rats. These effects were supported by the evidence that stressed SwHi rats showed significantly lower sucrose intake overall from that of non-stressed SwHi rats, $F(1,9) = 6.773$, $p = .029$, stressed and non-stressed SwLo rats did not show significantly different sucrose intake, $F(1, 8) = .155$, $p = .704$, and stressed and non-stressed NS rats did not show significantly different sucrose intake, $F(1, 9) = .712$, $p = .421$.

Water Intake

HYPER rats. Figure 5 shows the water intake of both stressed and non-stressed female HYPER and female NS rats. The three factor repeated measures analysis of covariance as described in the methods section for female rats yielded no significant effects. The interaction of rat line X stress/no stress was not significant, $F(1, 18) = 2.633$, $p = .122$, which indicated that stress did not affect female HYPER and female NS rats differently. This result was supported by the evidence that stressed and non-stressed female HYPER rats did not show significantly different water intake, $F(1, 9) = 2.520$, $p = .147$, and stressed and non-stressed female NS rats did not show significantly different water intake, $F(1, 8) = .266$, $p = .620$.

Figure 6 shows the water intake of both stressed and non-stressed male HYPER and male NS rats. The three factor repeated measures analysis of covariance as described in the methods section for male rats yielded a difference between rat lines that approached significance, $F(1, 19) = 3.458$, $p = .079$, which indicated that male HYPER rats tended to show lower water intake from that of male NS rats during the CMS phase. A difference between stress groups approached significance, $F(1, 19) = 3.892$, $p = .063$, which indicated that stressed animals tended to show lower water intake from that of non-stressed animals. The interaction of rat line X stress/no stress was not significant, $F(1, 19) = .042$, $p = .840$, which indicated that stress did not affect male HYPER and male NS rats differently. However, stressed male HYPER rats tended to show lower water intake overall from that of non-stressed male HYPER rats, $F(1, 9) = 4.783$, $p = .057$, whereas stressed and non-stressed male NS rats did not show significantly different water intake, $F(1, 9) = .985$, $p = .347$.

SUS and RES rats. Figure 7 shows the water intake of both stressed and non-stressed male SUS, male RES, and male NS rats. The three factor repeated measures analysis of covariance as described in the methods section yielded a significant difference between rat lines, $F(2, 29) = 11.794$, $p = .000$, which indicated that different rat lines showed significantly different water intake during the CMS phase. Further analyses comparing pairs of rat lines revealed that SUS and NS rats did not show significantly different water intake, $F(1, 17) = 1.074$, $p = .315$, RES rats showed significantly lower water intake from that of NS rats, $F(1, 19) = 8.828$, $p = .008$, and SUS rats showed significantly higher water intake from that of RES rats, $F(1, 17) = 12.211$, $p = .003$. A difference between stress groups approached significance, $F(1, 27) = 3.016$, $p = .094$, which indicated that stressed animals tended to show lower water intake from that of non-stressed animals.

The interaction of rat line X stress/no stress approached significance, $F(2, 27) = 2.473$, $p = .103$, which indicated that different rat lines tended to be affected differently by stress. Further analyses comparing pairs of rat lines revealed that the interaction of rat line X stress/no stress between SUS and NS rats was not significant, $F(1, 17) = .127$, $p = .726$, which indicated that stress did not affect SUS and NS rats differently. The interaction of rat line X stress/no stress between RES and NS rats was significant, $F(1, 19) = 5.178$, $p = .035$, which indicated that RES rats' water intake was affected differently by stress from that of NS rats. The interaction of rat line X stress/no stress between SUS and RES rats was not significant, $F(1, 17) = 2.359$, $p = .143$, which indicated that stress did not affect SUS and RES rats differently. These results were supported by the evidence that stressed and non-stressed SUS rats did not show

significantly different water intake, F(1, 7) = 1.781, p = .224, stressed and non-stressed RES rats did not show significantly different water intake, F(1, 9) = .725, p = .416, and stressed NS rats showed significantly lower water intake overall from that of non-stressed NS rats, F(1, 9) = 5.408, p = .045.

SwHi and SwLo rats. Figure 8 shows the water intake of both stressed and non-stressed male SwHi, male SwLo, and male NS rats. The three factor repeated measures analysis of covariance as described in the methods section yielded a significant difference between stress groups, F(1, 28) = 8.865, p = .006, which indicated that stressed animals showed significantly lower water intake from that of non-stressed animals. The interaction of rat line X stress/no stress approached significance, F(2, 28) = 3.232, p = .055, which indicated that stress tended to affect different rat lines differently. Further analyses comparing pairs of rat lines revealed that the interaction of rat line X stress/no stress between SwHi and NS rats was significant, F(1, 19) = 5.285, p = .033, which indicated that SwHi rats' water intake was affected differently by stress from that of NS rats. The interaction of rat line X stress/no stress between SwLo and NS rats approached significance, F(1, 18) = 4.036, p = .060, which indicated that SwLo rats' water intake tended to be affected differently by stress from that of NS rats. The interaction of rat line X stress/no stress between SwHi and SwLo rats was not significant, F(1, 18) = .339, p = .568, which indicated that stress did not affect SwHi and SwLo rats differently. These results were supported by the evidence that stressed and non-stressed SwHi rats did not show significantly different water intake, F(1, 9) = .065, p = .805, stressed and non-stressed SwLo rats did not show significantly different water intake, F(1, 8) = .081, p =

.783, and stressed NS rats showed significantly lower water intake overall from that of non-stressed NS rats, $F(1, 9) = 13.919$, $p = .005$.

Total Fluid Intake

Figure 9 shows total fluid intake of both stressed and non-stressed female HYPER and female NS rats. Figure 10 shows total fluid intake of both stressed and non-stressed male HYPER and male NS rats. Figure 11 shows total fluid intake of both stressed and non-stressed male SUS, male RES, and male NS rats. Figure 12 shows total fluid intake of both stressed and non-stressed male SwHi, male SwLo, and male NS rats. In all rat lines total fluid intake was dominated by sucrose intake and thus the data for this measure are extremely similar to the data for the sucrose intake measure. Therefore, total fluid intake was not analyzed separately.

Preference for Sucrose

As described in the methods section, a ratio of water-intake-to-total-fluid-intake during the sucrose administration period was used as an indicator of how much water rats ingested during the sucrose administration period ("preference for water"). The more water rats ingested during this period (i.e., the larger was the “preference for water”), the less the rat preferred to drink sucrose. Thus, the larger the preference for water, the lower the preference for sucrose, and vice versa.

HYPER rats. Figure 13 shows preference for water of both stressed and non-stressed female HYPER and female NS rats. When all days in which rats were offered sucrose were analyzed by a three factor repeated measures analysis of covariance as described in the methods section, a significant interaction of rat line X stress/no stress was found, $F(1, 18) = 5.439$, $p = .032$, which indicated that female HYPER rats’

preference for water was affected differently by stress from that of female NS rats. This result was supported by the evidence that stressed female HYPER rats showed significantly higher preference for water (and therefore lower preference for sucrose) overall from that of non-stressed female HYPER rats, $F(1, 9) = 16.078$, $p = .003$, whereas stressed and non-stressed female NS rats did not show significantly different preference for water, $F(1, 8) = .859$, $p = .381$.

When only the first day of each exposure period was analyzed in a similar fashion, the interaction of rat line X stress/no stress approached significance, $F(1, 18) = 3.344$, $p = .084$, which indicated that female HYPER rats' preference for water tended to be affected differently by stress from that of female NS rats. However, stressed and non-stressed female HYPER rats did not show significantly different preference for water, $F(1, 9) = 2.895$, $p = .123$, and stressed and non-stressed female NS rats did not show significantly different preference for water, $F(1, 8) = .952$, $p = .358$.

Figure 14 shows preference for water of both stressed and non-stressed male HYPER and male NS rats. When all days in which rats were offered sucrose were analyzed by a three factor repeated measures analysis of covariance as described in the methods section, a significant difference between rat lines was found, $F(1, 19) = 8.497$, $p = .009$, which indicated that male HYPER rats showed significantly higher preference for water (and therefore lower preference for sucrose) from that of male NS rats during the CMS phase. A difference between stress groups approached significance, $F(1, 19) = 3.072$, $p = .096$, which indicated that stressed male animals tended to show higher preference for water (and therefore lower preference for sucrose) from that of non-stressed male animals. The interaction of rat line X stress/no stress approached

significance, $F(1, 19) = 4.004$, $p = .060$, which indicated that male HYPER rats' preference for water tended to be affected differently by stress from that of male NS rats. This result was supported by the evidence that stressed male HYPER rats tended to show higher preference for water (and therefore lower preference for sucrose) overall from that of non-stressed male HYPER rats, $F(1, 9) = 4.234$, $p = .070$, whereas stressed and non-stressed NS rats did not show significantly different preference for water, $F(1, 9) = .001$, $p = .973$.

When only the first day of each exposure period was analyzed in a similar fashion, the significant difference between rat lines remained, $F(1, 19) = 11.998$, $p = .003$. There was a significant difference between stress groups, $F(1, 19) = 20.573$, $p = .000$, which indicated that stressed animals showed significantly higher preference for water (and therefore lower preference for sucrose) from that of non-stressed animals. The interaction of rat line X stress was significant, $F(1, 19) = 5.405$, $p = .031$, which indicated that male HYPER rats' preference for water was affected differently by stress from that of male NS rats. This result was supported by the evidence that stressed male HYPER rats showed significantly higher preference for water (and therefore lower preference for sucrose) overall from that of non-stressed male HYPER rats, $F(1, 9) = 14.965$, $p = .004$, whereas stressed and non-stressed male NS rats did not show significantly different preference for water, $F(1, 9) = 3.106$, $p = .112$.

SUS and RES rats. Figure 15 shows the preference for water of both stressed and non-stressed male SUS, male RES, and male NS rats. When all days in which rats were offered sucrose were analyzed by a three factor repeated measures analysis of covariance as described in the methods section, a significant difference between rat lines was found,

$F(2, 27) = 8.970$, $p = .001$, which indicated that different rat lines showed significantly different preference for water during the CMS phase. Further analyses comparing pairs of rat lines revealed that SUS and NS rats did not show significantly different preference for water, $F(1, 17) = .296$, $p = .593$, RES rats showed significantly higher preference for water (and therefore lower preference for sucrose) from that of NS rats, $F(1, 19) = 7.293$, $p = .014$, and SUS rats showed significantly lower preference for water (and therefore higher preference for sucrose) from that of RES rats, $F(1, 17) = 9.343$, $p = .007$. The interaction of rat line X stress/no stress was not significant, $F(2, 27) = .552$, $p = .599$, which indicated that stress did not affect different rat lines differently. This result was supported by the evidence that stressed SUS rats tended to show higher preference for water (and thus lower preference for sucrose) overall from that of non-stressed SUS rats, $F(1, 7) = 4.701$, $p = .067$, stressed and non-stressed RES rats did not show significantly different preference for water, $F(1, 9) = .158$, $p = .700$, and stressed and non-stressed NS rats did not show significantly different preference for water, $F(1, 9) = 2.030$, $p = .188$.

When only the first day of each exposure period was analyzed in a similar fashion, the significant difference between rat lines remained, $F(2, 27) = 10.226$, $p = .000$. Further analyses comparing pairs of rat lines revealed that SUS and NS rats did not show significantly different preference for water, $F(1, 17) = .002$, $p = .963$, RES rats showed significantly higher preference for water (and therefore lower preference for sucrose) from that of NS rats, $F(1, 19) = 12.176$, $p = .002$, and SUS rats showed significantly lower preference for water (and therefore higher preference for sucrose) from that of RES rats, $F(1, 17) = 12.856$, $p = .002$. There was a significant difference between stress groups, $F(1, 27) = 12.809$, $p = .001$, which indicated that stressed animals

showed significantly higher preference for water (and therefore lower preference for sucrose) from that of non-stressed animals.

An interaction of rat line X stress/no stress approached significance, $F(2,27) = 3.229$, $p = .052$, which indicated that different rat lines' preference for water tended to be affected differently by stress. Further analyses comparing pairs of rat lines revealed that the interaction of rat line X stress/no stress between SUS and NS rats was not significant, $F(1, 17) = .020$, $p = .890$, which indicated that stress did not affect SUS and NS rats differently. The interaction of rat line X stress/no stress between RES and NS rats approached significance, $F(1, 19) = 4.167$, $p = .055$, which indicated that RES rats' preference for water tended to be affected differently by stress from that of NS rats. The interaction of rat line X stress/no stress between SUS and RES rats approached significance, $F(1, 17) = 3.737$, $p = .070$, which indicated that SUS rats' preference for water tended to be affected differently by stress from that of RES rats.

These results were supported by the evidence that stressed SUS rats tended to show higher preference for water (and therefore lower preference for sucrose) overall from that of non-stressed SUS rats, $F(1, 7) = 4.292$, $p = .077$, stressed RES rats showed significantly higher preference for water (and therefore lower preference for sucrose) overall from that of non-stressed RES rats, $F(1, 9) = 9.655$, $p = .013$, and stressed and non-stressed NS rats did not show significantly different preference for water, $F(1, 9) = 1.110$, $p = .320$.

SwHi and SwLo rats. Figure 16 shows the preference for water of both stressed and non-stressed male SwHi, male SwLo, and male NS rats. When all days in which rats were offered sucrose were analyzed by a three factor repeated measures analysis of

covariance comparing pairs of rat lines as described in the methods section, a significant difference between rat lines was found between SwHi and NS rats, $F(1, 19) = 5.220$, $p = .034$, which indicated that SwHi rats showed significantly lower preference for water (and thus higher preference for sucrose) from that of NS rats during the CMS phase. No other rat line effects were found. A significant interaction of rat line X stress/no stress was found, $F(2, 28) = 6.106$, $p = .006$, which indicated that different rat lines' preference for water was affected differently by stress. Further analyses comparing pairs of rat lines revealed a significant interaction of rat line X stress/no stress between SwHi and NS rats, $F(1, 19) = 14.610$, $p = .001$, which indicated that SwHi rats' preference for water was affected differently by stress from that of NS rats. The interaction of rat line X stress/no stress between SwLo and NS rats was not significant, $F(1, 18) = 1.430$, $p = .247$, which indicated that stress did not affect SwLo and NS rats differently. The interaction of rat line X stress/no stress between SwHi and SwLo rats was significant, $F(1, 18) = 4.956$, $p = .039$, which indicated that SwHi rats' preference for water was affected differently by stress from that of SwLo rats. These results were supported by the evidence that stressed SwHi rats showed significantly higher preference for water (and therefore lower preference for sucrose) overall from that of non-stressed SwHi rats, $F(1, 9) = 12.629$, $p = .006$, stressed and non-stressed SwLo rats did not show significantly different preference for water, $F(1, 8) = .326$, $p = .583$, and stressed NS rats tended to show lower preference for water (and therefore higher preference for sucrose) overall from that of non-stressed NS rats, $F(1, 9) = 4.687$, $p = .059$.

When only the first day of each exposure period was analyzed in a similar fashion, a difference between rat lines was found that approached significance, $F(2, 28) =$

2.766, p = .080, which indicated that different rat lines tended to show different preference for water during the CMS phase. Further analyses comparing pairs of rat lines revealed that SwHi rats showed significantly lower preference for water (and therefore higher preference for sucrose) from that of NS rats, F(1, 19) = 5.567, p = .029, SwLo and NS rats did not show significantly different preference for water, F(1, 18) = .474, p = .500, and SwHi and SwLo rats did not show significantly different preference for water, F(1, 18) = 2.842, p = .109.

An interaction of rat line X stress/no stress approached significance, F(2, 28) = 2.699, p = .085, which indicated that different rat lines' preference for water tended to be affected differently by stress. Further analyses comparing pairs of rat lines revealed that the interaction of rat line X stress/no stress between SwHi and NS rats was significant, F(1, 19) = 5.307, p = .033, which indicated that SwHi rats' preference for water was affected differently by stress from that of NS rats. The interaction of rat line X stress/no stress between SwLo and NS rats was not significant, F(1, 18) = .303, p = .589, which indicated that stress did not affect SwLo and NS rats differently. The interaction of rat line X stress/no stress between SwHi and SwLo rats approached significance, F(1, 18) = 3.095, p = .096, which indicated that SwHi rats' preference for water tended to be affected differently by stress from that of SwLo rats. These results were supported by the evidence that stressed SwHi rats showed significantly higher preference for water (and therefore lower preference for sucrose) overall from that of non-stressed SwHi rats, F(1, 9) = 11.694, p = .008, stressed and non-stressed SwLo rats did not show significantly different preference for water, F(1, 8) = .053, p = .824, and stressed and non-stressed NS rats did not show significantly different preference for water, F(1, 9) = .383, p = .551.

Food Intake

HYPER rats. Figure 17 shows the food intake of both stressed and non-stressed female HYPER and female NS rats. The three factor repeated measures analysis of covariance as described in the methods section for female rats yielded a significant difference between stress groups, $F(1, 19) = 10.566$, $p = .004$, which indicated that stressed female animals showed significantly lower food intake from that of non-stressed female animals. The interaction of rat line X stress/no stress was not significant, $F(1, 19) = .389$, $p = .540$, which indicated that stress did not affect female HYPER and female NS rats differently. However, stressed and non-stressed female HYPER rats did not show significantly different food intake, $F(1, 9) = 3.035$, $p = .115$, whereas stressed female NS rats showed significantly lower food intake overall from that of non-stressed female NS rats, $F(1, 9) = 5.445$, $p = .044$.

Figure 18 shows the food intake of both stressed and non-stressed male HYPER and male NS rats. The three factor repeated measures analysis of covariance as described in the methods section for male rats yielded a significant difference between rat lines, $F(1, 19) = 10.085$, $p = .005$, which indicated that male HYPER rats showed significantly lower food intake from that of male NS rats during the CMS phase. There was a significant difference between stress groups, $F(1, 19) = 7.395$, $p = .014$, which indicated that stressed male animals showed significantly lower food intake from that of non-stressed male animals. The interaction of rat line X stress/no stress was not significant, $F(1, 19) = .036, .852$, which indicated that stress did not affect male HYPER and male NS rats differently. However, stressed male HYPER rats showed significantly lower food intake overall from that of non-stressed male HYPER rats, $F(1, 9) = 7.579$, $p = .022$,

whereas stressed and non-stressed male NS rats did not show significantly different food intake, $F(1, 9) = 1.905$, $p = .201$.

SUS and RES rats. Figure 19 shows the food intake of both stressed and non-stressed male SUS, male RES, and male NS rats. The three factor repeated measures analysis of covariance as described in the methods section yielded a significant difference between rat lines, $F(2, 29) = 4.194$, $p = .025$, which indicated that different rat lines showed significantly different food intake during the CMS phase. Further analyses comparing pairs of rat lines revealed that SUS and NS rats did not show significantly different food intake, $F(1, 19) = 1.919$, $p = .182$, RES rats showed significantly lower food intake from that of NS rats, $F(1, 19) = 8.291$, $p = .010$, and SUS rats tended to show higher food intake from that of RES rats, $F(1, 19) = 4.063$, $p = .058$. There was a significant difference between stress groups, $F(1, 29) = 10.257$, $p = .003$, which indicated that stressed animals showed significantly lower food intake from that of non-stressed animals. The interaction effect of rat line X stress/no stress was not significant, $F(2, 29) = .993$, $p = .383$, which indicated that stress did not affect different rat lines differently. However, stressed SUS rats tended to show lower food intake overall from that of non-stressed SUS rats, $F(1, 9) = 3.409$, $p = .098$, stressed and non-stressed RES rats did not show significantly different food intake, $F(1, 9) = 1.032$, $p = .336$, and stressed NS rats showed significantly lower food intake overall from that of non-stressed NS rats, $F(1, 9) = 9.158$, $p = .014$.

SwHi and SwLo rats. Figure 20 shows the food intake of both stressed and non-stressed male SwHi, male SwLo, and male NS rats. The three factor repeated measures analysis of covariance comparing pairs of rat lines as described in the methods section

yielded a difference between SwHi and NS rats that approached significance, $F(1, 19) = 3.471$, $p = .078$, which indicated that SwHi rats tended to show higher food intake from that of NS rats. No other rat line effects were found. The interaction of rat line X stress/no stress was significant, $F(2, 28) = 7.353$, $p = .003$, which indicated that different rat lines reacted differently to stress. Further analyses comparing pairs of rat lines revealed a significant interaction of rat line X stress/no stress between SwHi and NS rats, $F(1, 19) = 11.568$, $p = .003$, which indicated that SwHi rats' food intake was affected differently by stress from that of NS rats. The interaction of rat line X stress/no stress between SwLo and NS rats approached significance, $F(1, 18) = 4.376$, $p = .051$, which indicated that SwLo rats' food intake tended to be affected differently by stress from that of NS rats. The interaction of rat line X stress/no stress between SwHi and SwLo rats was significant, $F(1, 18) = 4.657$, $p = .045$, which indicated that SwHi rats' food intake was affected differently by stress from that of SwLo rats. However, stressed and non-stressed SwHi rats did not show significantly different food intake, $F(1, 9) = 2.902$, $p = .123$, stressed and non-stressed SwLo rats did not show significantly different food intake, $F(1, 8) = .201$, $p = .666$, and stressed NS rats showed significantly lower food intake overall from that of non-stressed NS rats, $F(1, 9) = 7.898$, $p = .020$.

Dark Phase Motor Activity

HYPER rats. Figure 21 shows the dark phase motor activity of both stressed and non-stressed female HYPER and female NS rats. The three factor repeated measures analysis of covariance as described in the methods section for female rats yielded a significant difference between rat lines, $F(1, 19) = 16.871$, $p = .001$, which indicated that female HYPER rats showed significantly higher dark phase motor activity from that of

female NS rats during the CMS phase. Additionally, the interaction of rat line X stress/no stress approached significance, $F(1, 19) = 2.952$, $p = .102$, which indicated that stressed female HYPER rats' dark phase motor activity was affected differently by stress from that of female NS rats. This result was supported by the evidence that stressed and non-stressed female HYPER rats did not show significantly different dark phase motor activity, $F(1, 9) = .642$, $p = .444$, whereas stressed female NS rats tended to show higher dark phase motor activity overall from that of non-stressed female NS rats, $F(1, 9) = 3.961$, $p = .078$.

Figure 22 shows the dark phase motor activity of both stressed and non-stressed male HYPER and male NS rats. The three factor repeated measures analysis of covariance as described in the methods section for male rats yielded a significant difference between rat lines, $F(1, 19) = 5.622$, $p = .028$, which indicated that male HYPER rats showed significantly higher dark phase motor activity from that of male NS rats during the CMS phase. There was a significant difference between stress groups, $F(1, 19) = 4.264$, $p = .053$, which indicated that stressed animals showed significantly lower dark phase activity from that of non-stressed animals. The interaction of rat line X stress/no stress did not approach significance, $F(1, 19) = .488$, $p = .493$, which indicated that stress did not affect male HYPER and male NS rats differently. However, stressed and non-stressed male HYPER rats did not show significantly different dark phase motor activity, $F(1, 9) = 2.483$, $p = .150$, whereas stressed male NS rats tended to show lower dark phase motor activity from that of non-stressed male NS rats, $F(1, 9) = 4.043$, $p = .075$.

SUS and RES rats. Figure 23 shows the dark phase motor activity of both stressed and non-stressed male SUS, male RES, and male NS rats. The three factor repeated measures analysis of covariance as described in the methods section yielded a significant difference between rat lines, $F(2, 29) = 11.655$, $p = .000$, which indicated that different rat lines showed significantly different dark phase motor activity during the CMS phase. Further analyses comparing pairs of rat lines revealed that SUS rats showed significantly higher dark phase motor activity from that of NS rats, $F(1, 19) = 4.451$, $p = .048$, RES rats showed significantly lower dark phase motor activity from that of NS rats, $F(1,19) = 8.017$, $p = .011$, and SUS rats showed significantly higher dark phase motor activity from that of RES rats, $F(1, 19) = 21.653$, $p = .000$. There was a significant difference between stress groups, $F(1, 29) = 13.187$, $p = .001$, which indicated that stressed animals showed significantly lower dark phase motor activity from that of non-stressed animals.

Additionally, the interaction of rat line X stress/no stress was significant, $F(2, 29) = 3.908$, $p = .031$, which indicated that different rat lines' dark phase motor activity was affected differently by stress. Further analyses comparing pairs of rat lines revealed that the interaction of rat line X stress/no stress between SUS and NS rats was not significant, $F(1, 19) = 1.555$, $p = .228$, which indicated that stress did not affect SUS and NS rats differently. The interaction of rat line X stress/no stress between RES and NS rats was not significant, $F(1, 19) = 2.635$, $p = .121$, which indicated that stress did not affect RES and NS rats differently. The interaction of rat line X stress/no stress between SUS and RES rats was significant, $F(1, 19) = 6.871$, $p = .017$, which indicated that SUS rats' dark phase motor activity was affected differently by stress from that of RES rats.

These results were supported by the evidence that stressed SUS rats showed significantly lower dark phase motor activity overall from that of non-stressed SUS rats, $F(1, 9) = 11.067$, $p = .009$, stressed and non-stressed RES rats did not show significantly different dark phase motor activity, $F(1, 9) = .020$, $p = .889$, and stressed NS rats tended to show lower dark phase motor activity overall from that of non-stressed NS rats, $F(1, 9) = 4.844$, $p = .055$.

SwHi and SwLo rats. Figure 24 shows the dark phase motor activity of both stressed and non-stressed male SwHi, male SwLo, and male NS rats. The three factor repeated measures analysis of covariance comparing pairs of rat lines as described in the methods section yielded a difference between SwHi and NS rats that approached significance, $F(1, 19) = 3.521$, $p = .076$, which indicated that SwHi rats tended to show higher dark phase motor activity from that of NS rats. No other rat line effects were found. A significant difference between stress groups was found, $F(1, 28) = 36.245$, $p = .000$, which indicated that stressed animals showed significantly lower dark phase motor activity from that of non-stressed animals. Additionally, the interaction of rat line X stress/no stress was significant, $F(2,28) = 5.066$, $p = .013$, which indicated that the effect of stress on dark phase motor activity differed for different rat lines. Further analyses comparing pairs of rat lines revealed that the interaction of rat line X stress/no stress between SwHi and NS rats was significant, $F(1, 19) = 9.809$, $p = .005$, which indicated that SwHi rats' dark phase motor activity was affected differently by stress from that of NS rats. The interaction of rat line X stress/no stress between SwLo and NS rats approached significance, $F(1, 18) = 3.347$, $p = .084$, which indicated that stressed SwLo rats' dark phase motor activity was affected differently by stress from that of NS rats.

The interaction of rat line X stress/no stress between SwHi and SwLo rats was not significant, $F(1, 18) = 1.910$, $p = .184$, which indicated that stress did not affect SwHi and SwLo rats differently.

These results were supported by the evidence that stressed SwHi rats showed significantly lower dark phase motor activity overall from that of non-stressed SwHi rats, $F(1, 9) = 32.712$, $p = .000$, stressed SwLo rats showed significantly lower dark phase motor activity overall from that of non-stressed SwLo rats, $F(1, 8) = 12.396$, $p = .008$, and stressed and non-stressed NS rats did not show significantly different dark phase motor activity, $F(1, 9) = 1.069$, $p = .328$.

Light Phase Motor Activity

HYPER rats. Figure 25 shows the light phase motor activity of both stressed and non-stressed female HYPER and female NS rats. The three factor repeated measures analysis of covariance as described in the methods section for female rats yielded a significant difference between rat lines, $F(1, 19) = 4.876$, $p = .040$, which indicated that female HYPER rats showed significantly lower light phase motor activity from that of female NS rats during the CMS phase. A difference between stress groups approached significance, $F(1, 19) = 3.459$, $p = .078$, which indicated that stressed female animals tended to show higher light phase motor activity from that of non-stressed female animals. The interaction of rat line X stress/no stress was not significant, $F(1, 19) = .336$, $p = .552$, which indicated that stress did not affect female HYPER and female NS rats differently. This result was supported by the evidence that stressed and non-stressed female HYPER rats did not show significantly different light phase motor activity, $F(1,$

9) = .939, p = .358, and stressed and non-stressed NS rats did not show significantly different light phase motor activity, F(1, 9) = 2.219, p = .170.

Figure 26 shows the light phase motor activity of both stressed and non-stressed male HYPER and male NS rats. The three factor repeated measures analysis of covariance as described in the methods section for male rats yielded a significant difference between rat lines, F(1, 19) = 6.618, p = .019, which indicated that male HYPER rats showed significantly higher light phase motor activity from that of male NS rats during the CMS phase. The interaction of rat line X stress/no stress was not significant, F(1, 19) = .152, p = .701, which indicated that stress did not affect male HYPER and male NS rats differently. This result was supported by the evidence that stressed and non-stressed male HYPER rats did not show significantly different light phase motor activity, F(1, 9) = .502, p = .496, and stressed and non-stressed NS rats did not show significantly different light phase motor activity, F(1, 9) = .072, p = .794.

SUS and RES rats. Figure 27 shows the light phase motor activity of both stressed and non-stressed male SUS, male RES, and male NS rats. The three factor repeated measures analysis of covariance as described in the methods section yielded a significant difference between rat lines, F(2, 29) = 6.165, p = .006, which indicated that different rat lines showed significantly different light phase motor activity during the CMS phase. Further analyses comparing pairs of rat lines revealed that SUS rats showed significantly higher light phase motor activity from that of NS rats, F(1, 19) = 12.688, p = .002, RES rats tended to show higher light phase motor activity from that of NS rats, F(1, 19) = 3.574, p = .074, and SUS and RES rats did not show significantly different light phase motor activity, F(1, 19) = 2.398, p = .138.

The interaction of rat line X stress/no stress was significant, $F(2, 29) = 5.224$, $p = .012$, which indicated that different rat lines' light phase motor activity was affected differently by stress. Further analyses comparing pairs of rat lines revealed that the interaction of rat line X stress/no stress between SUS and NS rats was significant, $F(1, 19) = 10.130$, $p = .005$, which indicated that SUS rats' light phase motor activity was affected differently by stress from that of NS rats. The interaction of rat line X stress/no stress between RES and NS rats was not significant, $F(1, 19) = 2.111$, $p = .163$, which indicated that stress did not affect RES and NS rats differently. The interaction of rat line X stress/no stress between SUS and RES rats was not significant, $F(1, 19) = 2.356$, $p = .141$, which indicated that stress did not affect SUS and RES rats differently.

These results were supported by the evidence that stressed SUS rats tended to show lower light phase motor activity overall from that of non-stressed SUS rats, $F(1, 9) = 3.594$, $p = .091$, stressed and non-stressed RES rats did not show significantly different light phase motor activity, $F(1, 9) = .000$, $p = .996$, and stressed NS rats tended to show higher light phase motor activity overall from that of non-stressed NS rats, $F(1, 9) = 4.835$, $p = .055$.

SwHi and SwLo rats. Figure 28 shows the light phase motor activity of both stressed and non-stressed male SwHi, male SwLo, and male NS rats. The three factor repeated measures analysis of covariance as described in the methods section yielded a significant difference between rat lines, $F(2, 28) = 6.037$, $p = .007$, which indicated that different rat lines showed significantly different light phase activity during the CMS phase. Further analyses comparing pairs of rat lines revealed that SwHi rats showed significantly higher light phase motor activity from that of NS rats, $F(1, 19) = 22.367$, $p =$

.000, SwLo and NS rats did not show significantly different light phase motor activity, $F(1, 18) = 1.547$, $p = .230$, and SwHi and SwLo rats did not show significantly different light phase motor activity, $F(1, 18) = 1.856$, $p = .190$. The effect of stress approached significance, $F(1, 28) = 3.899$, $p = .058$, which indicated that stressed animals tended to show lower light phase motor activity from that of non-stressed animals.

The interaction of rat line X stress/no stress approached significance, $F(2, 28) = 2.567$, $p = .095$, which indicated that different rat lines' light phase motor activity was affected differently by stress. Further analyses comparing pairs of rat lines showed that the interaction of rat line X stress/no stress between SwHi and NS rats approached significance, $F(1, 19) = 3.525$, $p = .076$, which indicated that SwHi rats' light phase motor activity tended to be affected differently from that of NS rats. The interaction of rat line X stress/no stress between SwLo and NS rats was not significant, $F(1, 18) = 1.420$, $p = .249$, which indicated that stress did not affect SwLo and NS rats differently. The interaction of rat line X stress/no stress between SwHi and SwLo rats was significant, $F(1, 18) = 4.427$, $p = .050$, which indicated SwHi rats' light phase motor activity was affected differently by stress from that of NS rats.

However, stressed and non-stressed SwHi rats did not show significantly different light phase motor activity, $F(1, 9) = .988$, $p = .346$, stressed SwLo rats showed significantly lower light phase motor activity from that of non-stressed SwLo rats, $F(1, 8) = 18.334$, $p = .003$, and stressed and non-stressed NS rats did not show significantly different light phase motor activity, $F(1, 9) = 2.328$, $p = .161$.

Discussion

The selectively-bred rat lines used in this study did indeed differ in regards to the ways in which they reacted to CMS, especially when compared to NS rats. Perhaps the most important results to note in this study are the observed effects on preference for sucrose in each rat line since loss of preference for sucrose is hypothesized to indicate a state of anhedonia in rats (Willner, 1997). Many of the selectively-bred rat lines showed lower sucrose intake and lower preference for sucrose when stressed, in stark contrast to NS rats which did not show significantly different sucrose intake or preference for sucrose.

Although no rat line ever exhibited a loss of preference for sucrose when stressed (where a loss of preference for sucrose would be indicated by a preference for sucrose of 50% or less), there were nonetheless rat lines which exhibited lower preference for sucrose when stressed. HYPER rats, both females and males, and SwHi rats showed lower preference for sucrose when stressed. SUS rats also tended to show lower preference for sucrose when stressed. RES rats also showed lower preference for sucrose when stressed but this effect only lasted for the first day of each sucrose administration period. SwLo rats were the only selectively bred line that did not show any effect on preference for sucrose when stressed. In contrast, female and male NS rats did not show any effect on preference for sucrose when stressed, and one group of male NS rat actually tended to show higher preference for sucrose when stressed. Furthermore, the fact none of the four NS groups used in the study ever showed a decrease in preference for sucrose when stressed suggests that the effects seen in the study are not due to random variability in the outcome of CMS.

The results for sucrose intake mostly mirrored the results for the measure of preference for sucrose in each rat line, with the exceptions being that SUS rats did not show an effect on sucrose intake when stressed and neither did male NS rats. In addition, a striking observation was that all rat lines always showed the greatest amount of sucrose intake on the first day of each sucrose administration period.

The reduction in sucrose intake and preference in RES, SwHi, and female HYPER rats when stressed could not be explained by a reduction in caloric intake as neither food nor water intake changed significantly overall in these rat lines when stressed. This is an important finding because it has been suggested in the past that the reduction in preference for sucrose is due to a reduction in the caloric intake of rats (Hatcher et al., 1997; Reid et al., 1997). While SUS rats tended to show lower food intake when stressed, neither water nor sucrose intake was affected which indicated that fluid intake was not affected and thus the trend for stressed SUS rats to show lower preference for sucrose was a real reduction in preference. While male NS rats did show significantly lower food and water intake when stressed, sucrose intake was unaffected which suggests that while their caloric intake may have been lower, the fact that they still drank the same amount of sucrose as before is indicative of a true increase in preference for sucrose. Male HYPER rats were the only rat line in which lower sucrose intake was also accompanied by lower food intake and a tendency to show lower water intake when stressed.

Furthermore, it has been claimed that food deprivation as a component of CMS is sufficient to result in a decrease in sucrose intake, hypothesized to be due to decreased caloric intake as a result of decreased metabolism (Forbes et al., 1996; Hatcher et al.,

1997; Reid et al., 1997). It has also been found that increasing the time period between food deprivation and administration of sucrose to over 24 hours is enough to eliminate the decrease in sucrose intake as a result of CMS (Hagan & Hatcher, 1997; Hatcher et al., 1997). Relative to the current study food deprivation was always administered at least 96 hours before the start of a sucrose administration period, and thus the effects of stress (CMS) on sucrose intake and/or preference for sucrose observed in this study could also not be attributed to the use of the food deprivation stressor.

Effects on motor activity in response to stress also varied between rat lines. Some lines showed lower activity during both the light and dark phases when stressed (SUS and SwLo) while others showed no effects during either the light or dark phases when stressed (HYPER and RES). Other lines showed lower dark phase motor activity when stressed but no effect during the light phase (SwHi and male NS). Female NS rats were unique in this study in that they were the only rat line that had a tendency to show higher dark phase motor activity when stressed, although there was no effect on light phase activity.

An interesting qualitative observation was made for the dark phase motor activity of some rat lines. During sucrose administration periods, dark phase motor activity would sometimes be observed to sharply increase, or "spike," for the duration of the sucrose administration period before returning to the level of activity normally observed for that rat line. This spiking behavior, however, seemed to be suppressed by the CMS procedure. Activity spikes were observed during both experimental sucrose administration periods in non-stressed female NS rats but in stressed female NS rats were only observed during the last experimental sucrose administration period. Activity spikes

were observed during both experimental sucrose administration periods in non-stressed male NS rats but in stressed male NS rats were not observed during either experimental sucrose administration phase. Activity spikes were observed during both experimental sucrose administration periods in non-stressed female HYPER rats but in stressed female HYPER rats were only observed during the last experimental sucrose administration period. Activity spikes were observed during both experimental sucrose administration periods in non-stressed SwHi rats but in stressed SwHi rats were not observed during either experimental phase. Activity spikes were not observed in any other rat line.

The lack of effects of CMS on dark activity in the HYPER rats used in this study should not be construed as evidence that HYPER rats do not show hyperactivity in response to stress. In previous studies, the stressors that elicited hyperactivity in HYPER rats were acute stressors (Weiss et al., 2008). It is thus possible that the hyperactivity regularly observed in HYPER rats is only elicited by acute stressors and not chronic stressors such as produced by the CMS procedure.

One limitation of this study was that non-stressed groups in Experiments 2 and 4 were comprised of only four rats from each rat line. It is possible that effects that failed to reach significance in these experiments may have reached significance had the number of non-stressed rats matched the number of stressed rats, even if the trends observed in these experiments had remained identical. Another issue with this study was that the stressors used were more severe than those used in other CMS studies (Willner, 2005). However, it would appear from other studies that CMS is most effective when it incorporates severe stressors, to the point that some animals may die (e.g., Streklova et al., 2004). Furthermore, Katz's original chronic stress paradigm also used severe

stressors (Katz, 1981). Thus, the incorporation of severe stressors should not necessarily be taken as evidence that the results from this study cannot be generalized.

The findings of this study have implications for the selection of animals for use in CMS experiments. The genetic makeup and behavioral predispositions of a line of animals do influence the outcome of CMS. Some rat lines may be more resistant to a decrease in preference for sucrose whereas others may be more susceptible to a decrease in preference for sucrose. Some rat lines may even be liable to produce a paradoxical increase in preference for sucrose in response to CMS. Thus, it is important that researchers consider the known behavior of a rat line before using it in a CMS experiment. Researchers may especially want to avoid rat lines which are not selectively-bred since their behavior may not be easily categorized, especially since genetic drift in a particular animal supplier or lab's stock of animals could result in rats with subtle but important behavioral differences from those of other animal suppliers or labs, even if they are of the same rat line.

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Table 1

Chronic Mild Stress (CMS) Regime

Stressor	Abbreviation	Description
Restraint	Res	Rats were individually restrained for two hours
Food Deprivation	FDep	Food was removed from cages for 24 hours on days 9, 20, and 27 (water *ad libitum*).
Overnight Illumination	NiLi	Lights were left on overnight thus extending the rats light phase to 24 hours.
White Noise	WN	Rats were subjected to one hour of 95 dB white noise.
Foot Shock	Shk	One hour of foot shock delivered at 1.0 to 1.5 mA.
Bedding Switch	BedS	Rats were placed in other rats cages for 48 hours. Wet bedding was applied the day after.
Wet Bedding	WetB	The bedding of each cage was soaked with 450 ± 5 mL of water and changed after 24 hours.
Forced Swim	Swim	Rats were placed in a shallow tank of water at 78°F for 15 minutes.
None	None	No stressors administered

Figure Captions

Figure 1. Upper graphs show the average sucrose intake and SEM per day during each sucrose administration period of both stressed and non-stressed female Non-selected (NS) and female HYPER rats. Lower graphs show the difference in sucrose intake between non-stressed and stressed rats (i.e., the mean sucrose intake for stressed rats minus the mean sucrose intake for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. Days labeled "BL" denote days during the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 2. Upper graphs show the average sucrose intake and SEM per day during each sucrose administration period of both stressed and non-stressed male Non-selected (NS) and male HYPER rats. Lower graphs show the difference in sucrose intake between non-stressed and stressed rats (i.e., the mean sucrose intake for stressed rats minus the mean sucrose intake for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. Days labeled "BL" denote days during the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 3. Upper graphs show the average sucrose intake and SEM per day during each sucrose administration period of both stressed and non-stressed male Non-selected (NS), male SUS, male RES rats. Lower graphs show the difference in sucrose intake between non-stressed and stressed rats (i.e., the mean sucrose intake for stressed rats minus the mean sucrose intake for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1.

Days labeled “BL” denote days during the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 4. Upper graphs show the average sucrose intake and SEM per day during each sucrose administration period of both stressed and non-stressed male Non-selected (NS), male SwHi, male SwLo rats. Lower graphs show the difference in sucrose intake between non-stressed and stressed rats (i.e., the mean sucrose intake for stressed rats minus the mean sucrose intake for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. Days labeled “BL” denote days during the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 5. Upper graphs show the average water intake and SEM per day of both stressed and non-stressed female Non-selected (NS) and female HYPER rats. Lower graphs show the difference in water intake between non-stressed and stressed rats (i.e., the mean water intake for stressed rats minus the mean water intake for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. “BL” denotes beginning of the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 6. Upper graphs show the average water intake and SEM per day of both stressed and non-stressed male Non-selected (NS) and male HYPER rats. Lower graphs show the difference in water intake between non-stressed and stressed rats (i.e., the mean water intake for stressed rats minus the mean water intake for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the

abbreviations used see Table 1. "BL" denotes beginning of the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 7. Upper graphs show the average water intake and SEM per day of both stressed and non-stressed male Non-selected (NS), male SUS, male RES rats. Lower graphs show the difference in water intake between non-stressed and stressed rats (i.e., the mean water intake for stressed rats minus the mean water intake for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. "BL" denotes beginning of the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 8. Upper graphs show the average water intake and SEM per day of both stressed and non-stressed male Non-selected (NS), male SwHi, male SwLo rats. Lower graphs show the difference in water intake between non-stressed and stressed rats (i.e., the mean water intake for stressed rats minus the mean water intake for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. "BL" denotes beginning of the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 9. Upper graphs show the average total fluid intake and SEM per day during each sucrose administration period of both stressed and non-stressed female Non-selected (NS) and female HYPER rats. Lower graphs show the difference in total fluid intake between non-stressed and stressed rats (i.e., the mean total fluid intake for stressed rats minus the mean total fluid intake for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1.

Days labeled “BL” denote days during the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 10. Upper graphs show the average total fluid intake and SEM per day during each sucrose administration period of both stressed and non-stressed male Non-selected (NS) and male HYPER rats. Lower graphs show the difference in total fluid intake between non-stressed and stressed rats (i.e., the mean total fluid intake for stressed rats minus the mean total fluid intake for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. Days labeled “BL” denote days during the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 11. Upper graphs show the average total fluid intake and SEM per day during each sucrose administration period of both stressed and non-stressed male Non-selected (NS), male SUS, male RES rats. Lower graphs show the difference in total fluid intake between non-stressed and stressed rats (i.e., the mean total fluid intake for stressed rats minus the mean total fluid intake for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. Days labeled “BL” denote days during the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 12. Upper graphs show the average total fluid intake and SEM per day during each sucrose administration period of both stressed and non-stressed male Non-selected (NS), male SwHi, male SwLo rats. Lower graphs show the difference in total fluid intake between non-stressed and stressed rats (i.e., the mean total fluid intake for stressed rats minus the mean total fluid intake for non-stressed rats). X-axis labels indicate which

stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. Days labeled "BL" denote days during the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 13. Upper graphs show the average preference for water and SEM per day during each sucrose administration period of both stressed and non-stressed female Non-selected (NS) and female HYPER rats. Lower graphs show the difference in preference for water between non-stressed and stressed rats (i.e., the preference for water for stressed rats minus the preference for water for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. Days labeled "BL" denote days during the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 14. Upper graphs show the average preference for water and SEM per day during each sucrose administration period of both stressed and non-stressed male Non-selected (NS) and male HYPER rats. Lower graphs show the difference in preference for water between non-stressed and stressed rats (i.e., the preference for water for stressed rats minus the preference for water for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. Days labeled "BL" denote days during the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 15. Upper graphs show the average preference for water and SEM per day during each sucrose administration period of both stressed and non-stressed male Non-selected (NS), male SUS, male RES rats. Lower graphs show the difference in preference for water between non-stressed and stressed rats (i.e., the preference for water for stressed

rats minus the preference for water for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. Days labeled "BL" denote days during the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 16. Upper graphs show the average preference for water and SEM per day during each sucrose administration period of both stressed and non-stressed male Non-selected (NS), male SwHi, male SwLo rats. Lower graphs show the difference in preference for water between non-stressed and stressed rats (i.e., the preference for water for stressed rats minus the preference for water for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. Days labeled "BL" denote days during the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 17. Upper graphs show the average food intake and SEM per day of both stressed and non-stressed female Non-selected (NS) and female HYPER rats. Lower graphs show the difference in food intake between non-stressed and stressed rats (i.e., the mean food intake for stressed rats minus the mean food intake for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. "BL" denotes beginning of the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 18. Upper graphs show the average food intake and SEM per day of both stressed and non-stressed male Non-selected (NS) and male HYPER rats. Lower graphs show the difference in food intake between non-stressed and stressed rats (i.e., the mean food intake for stressed rats minus the mean food intake for non-stressed rats). X-axis labels

indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. "BL" denotes beginning of the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 19. Upper graphs show the average food intake and SEM per day of both stressed and non-stressed male Non-selected (NS), male SUS, male RES rats. Lower graphs show the difference in food intake between non-stressed and stressed rats (i.e., the mean food intake for stressed rats minus the mean food intake for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. "BL" denotes beginning of the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 20. Upper graphs show the average food intake and SEM per day of both stressed and non-stressed male Non-selected (NS), male SwHi, male SwLo rats. Lower graphs show the difference in food intake between non-stressed and stressed rats (i.e., the mean food intake for stressed rats minus the mean food intake for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. "BL" denotes beginning of the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 21. Upper graphs show the average dark phase ambulatory activity and SEM per day of both stressed and non-stressed female Non-selected (NS) and female HYPER rats. Lower graphs show the difference in dark phase ambulatory activity between non-stressed and stressed rats (i.e., the mean dark phase ambulatory activity for stressed rats minus the mean dark phase ambulatory activity for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the

abbreviations used see Table 1. "BL" denotes beginning of the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 22. Upper graphs show the average dark phase ambulatory activity and SEM per day of both stressed and non-stressed male Non-selected (NS) and male HYPER rats. Lower graphs show the difference in dark phase ambulatory activity between non-stressed and stressed rats (i.e., the mean dark phase ambulatory activity for stressed rats minus the mean dark phase ambulatory activity for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. "BL" denotes beginning of the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 23. Upper graphs show the average dark phase ambulatory activity and SEM per day of both stressed and non-stressed male Non-selected (NS), male SUS, male RES rats. Lower graphs show the difference in dark phase ambulatory activity between non-stressed and stressed rats (i.e., the mean dark phase ambulatory activity for stressed rats minus the mean dark phase ambulatory activity for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. "BL" denotes beginning of the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

Figure 24. Upper graphs show the average dark phase ambulatory activity and SEM per day of both stressed and non-stressed male Non-selected (NS), male SwHi, male SwLo rats. Lower graphs show the difference in dark phase ambulatory activity between non-stressed and stressed rats (i.e., the mean dark phase ambulatory activity for stressed rats minus the mean dark phase ambulatory activity for non-stressed rats). X-axis labels

indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. "BL" denotes beginning of the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

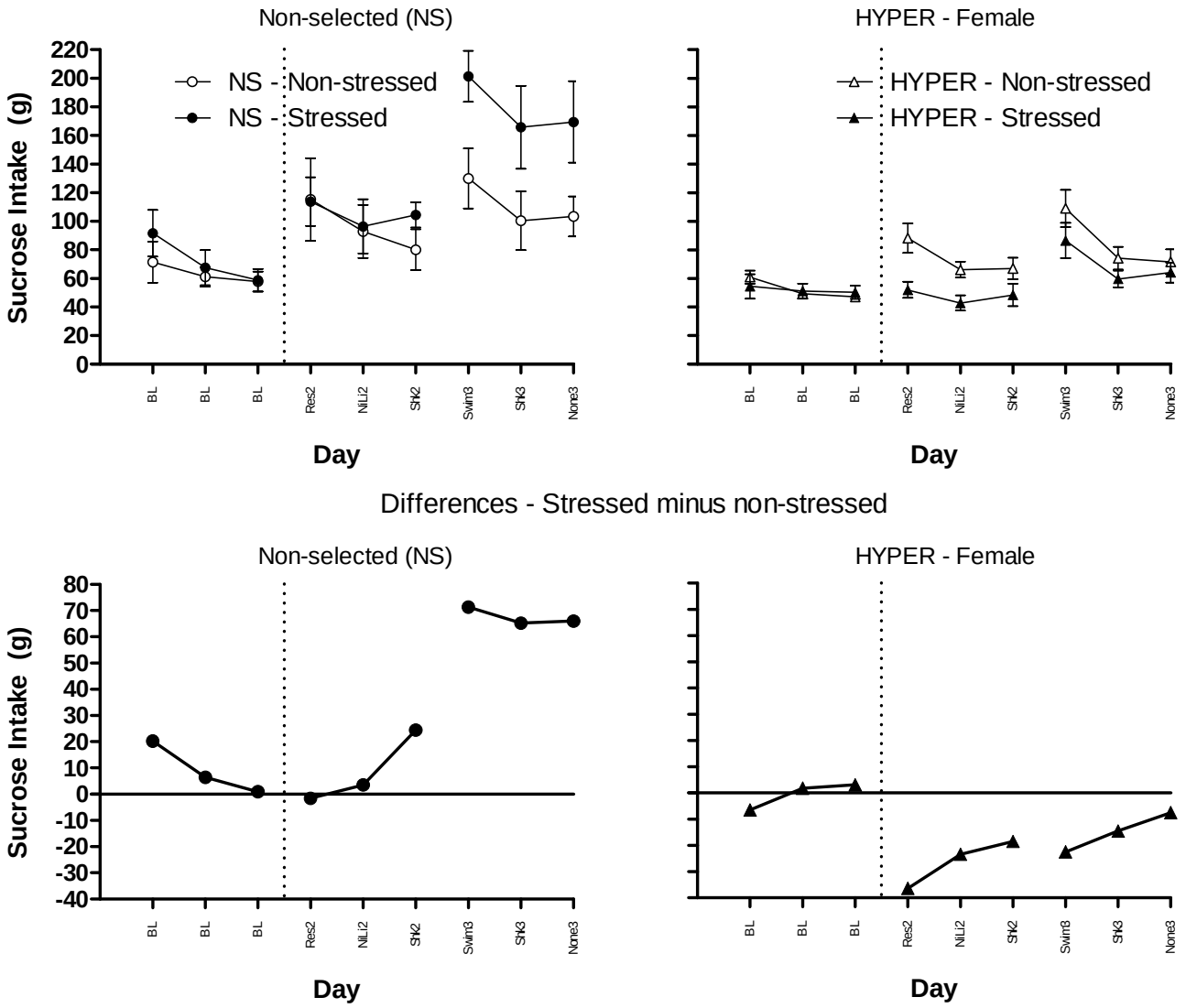
Figure 25. Upper graphs show the average light phase ambulatory activity and SEM per day of both stressed and non-stressed female Non-selected (NS) and female HYPER rats. Lower graphs show the difference in light phase ambulatory activity between non-stressed and stressed rats (i.e., the mean light phase ambulatory activity for stressed rats minus the mean light phase ambulatory activity for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. "BL" denotes beginning of the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

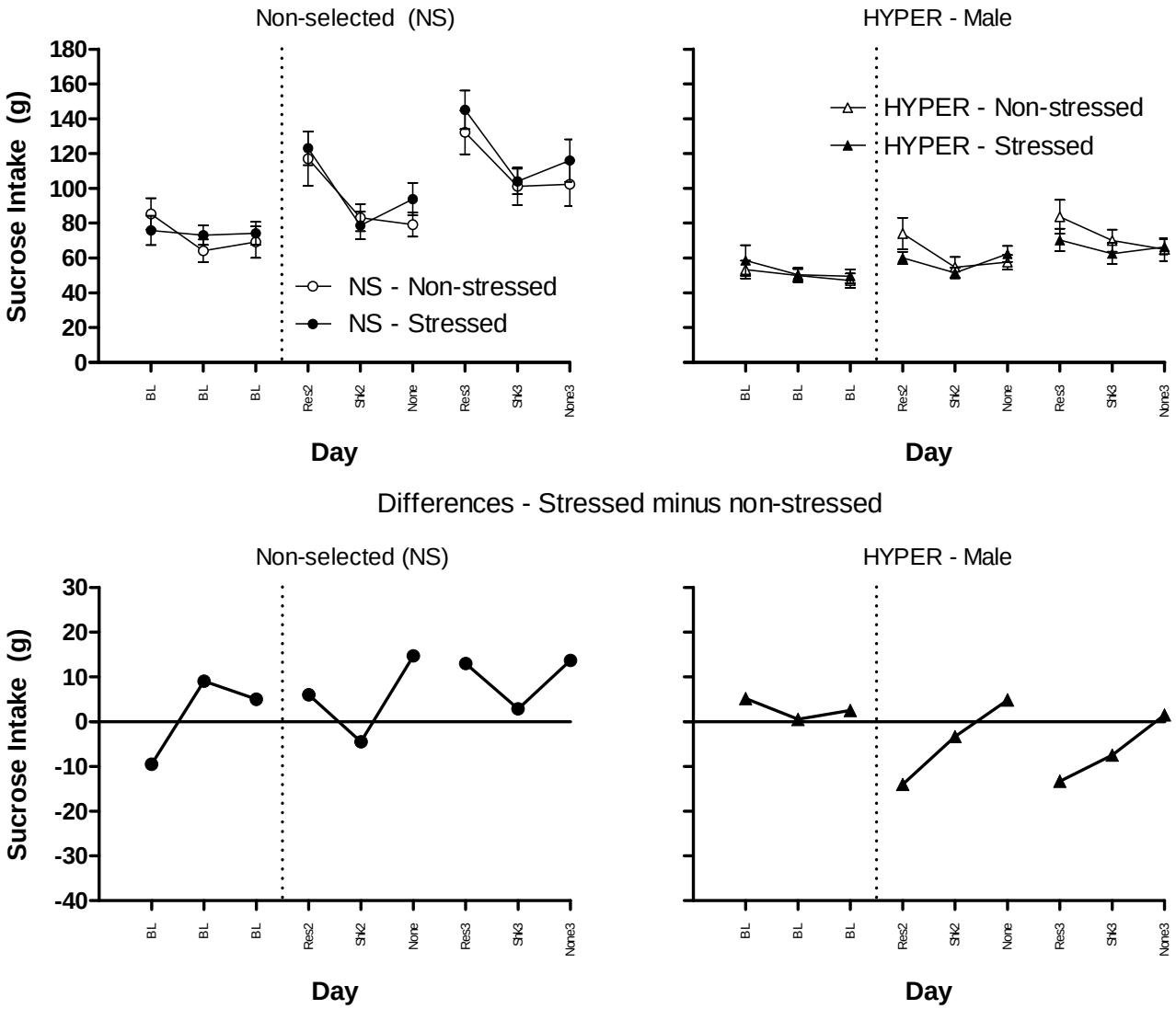
Figure 26. Upper graphs show the average light phase ambulatory activity and SEM per day of both stressed and non-stressed male Non-selected (NS) and male HYPER rats. Lower graphs show the difference in light phase ambulatory activity between non-stressed and stressed rats (i.e., the mean light phase ambulatory activity for stressed rats minus the mean light phase ambulatory activity for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. "BL" denotes beginning of the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

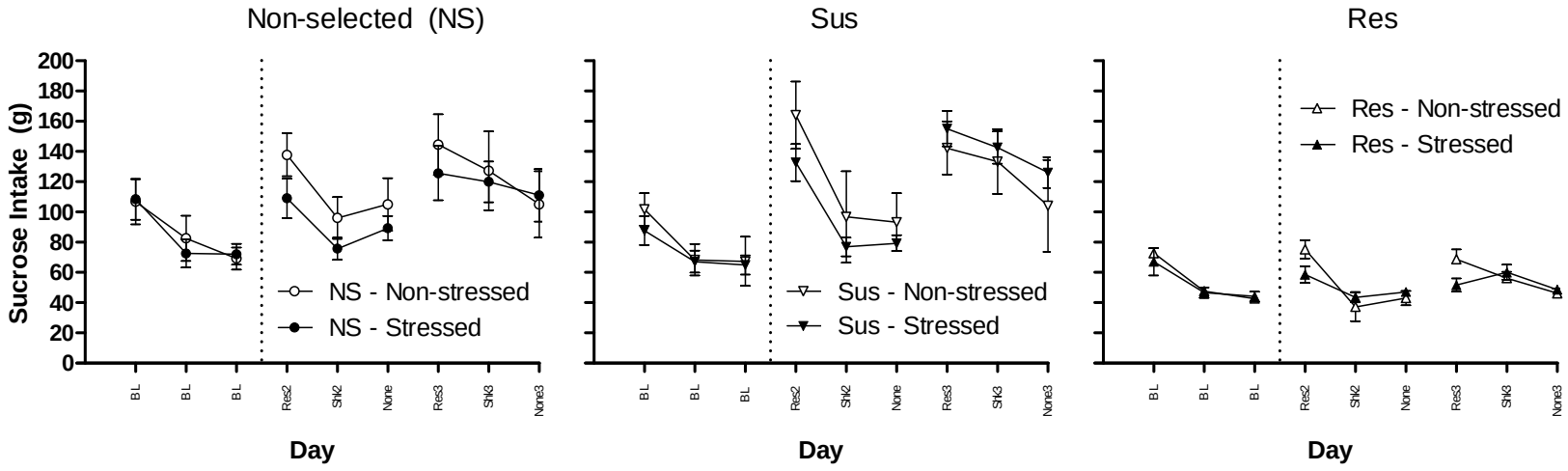
Figure 27. Upper graphs show the average light phase ambulatory activity and SEM per day of both stressed and non-stressed male Non-selected (NS), male SUS, male RES rats. Lower graphs show the difference in light phase ambulatory activity between non-stressed and stressed rats (i.e., the mean light phase ambulatory activity for stressed rats

minus the mean light phase ambulatory activity for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. "BL" denotes beginning of the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

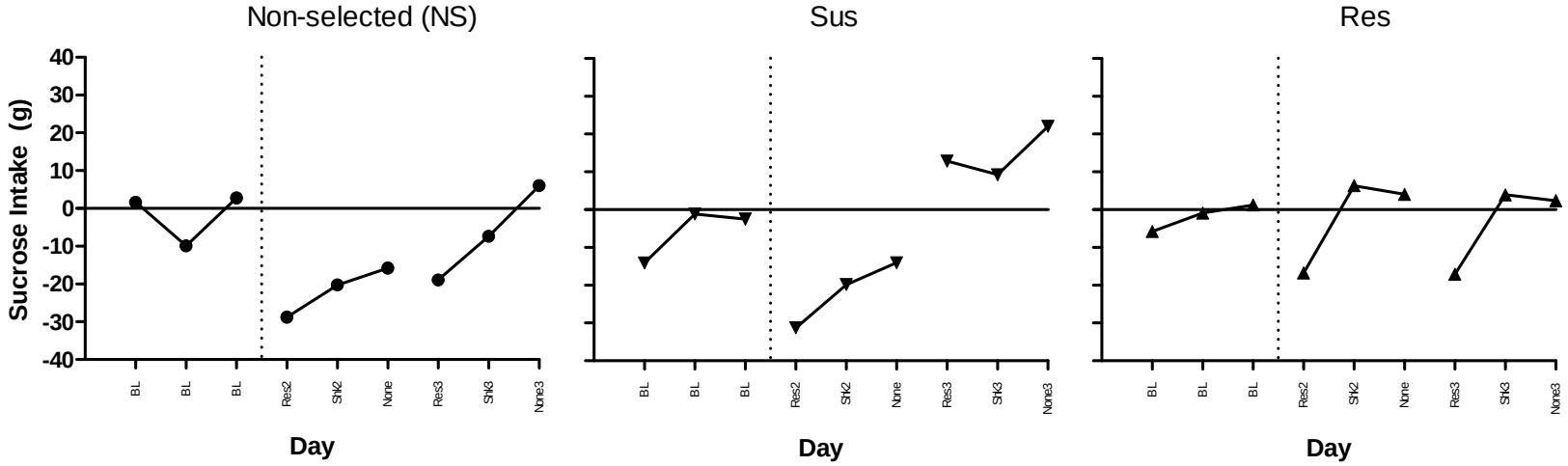
Figure 28. Upper graphs show the average light phase ambulatory activity and SEM per day of both stressed and non-stressed male Non-selected (NS), male SwHi, male SwLo rats. Lower graphs show the difference in light phase ambulatory activity between non-stressed and stressed rats (i.e., the mean light phase ambulatory activity for stressed rats minus the mean light phase ambulatory activity for non-stressed rats). X-axis labels indicate which stressor was implemented on each day. For an explanation of the abbreviations used see Table 1. "BL" denotes beginning of the baseline period. Vertical dotted line denotes beginning of CMS after baseline period.

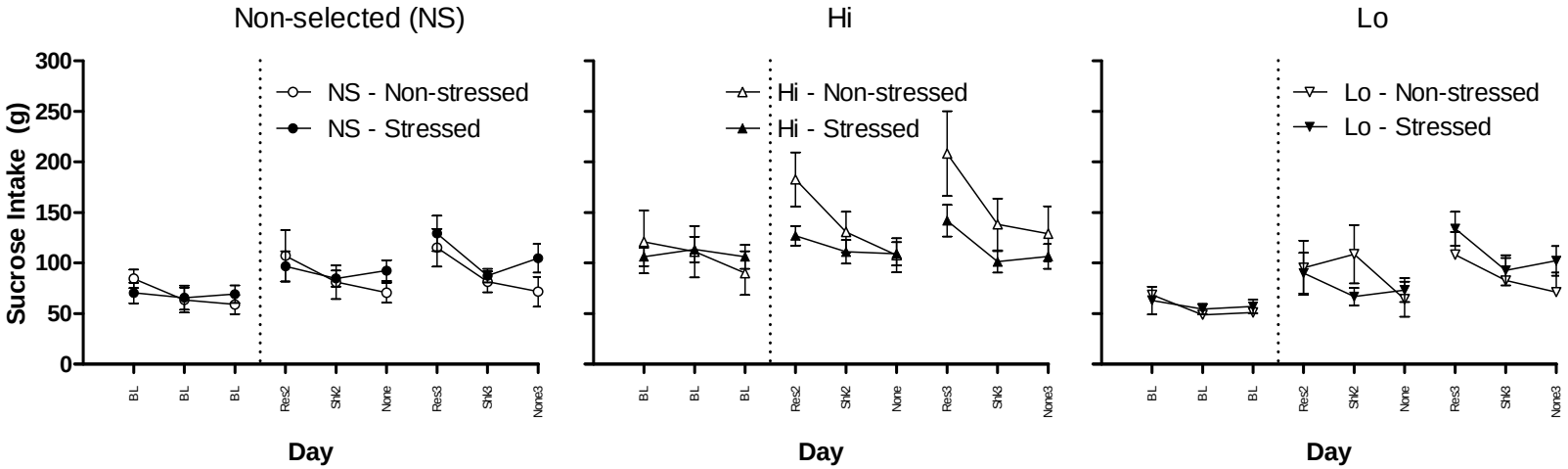

Non-selected (NS)
HYPER - Female
NS - Non-stressed
NS - Stressed
HYPER - Non-stressed
HYPER - Stressed
Sucrose Intake (g)
Day
BL
Res2
Nili2
Shk2
Swim3
Shk3
None3
Differences - Stressed minus non-stressed
Non-selected (NS)
HYPER - Female
Sucrose Intake (g)
Day


Non-selected (NS)
HYPER - Male
Sucrose Intake (g)
180
160
140
120
100
80
60
40
20
0
NS - Non-stressed
NS - Stressed
HYPER - Non-stressed
HYPER - Stressed
BL
BL
BL
Res2
Shk2
None
Res3
Shk3
None3
Day
Differences - Stressed minus non-stressed
30
20
10
0
-10
-20
-30
-40

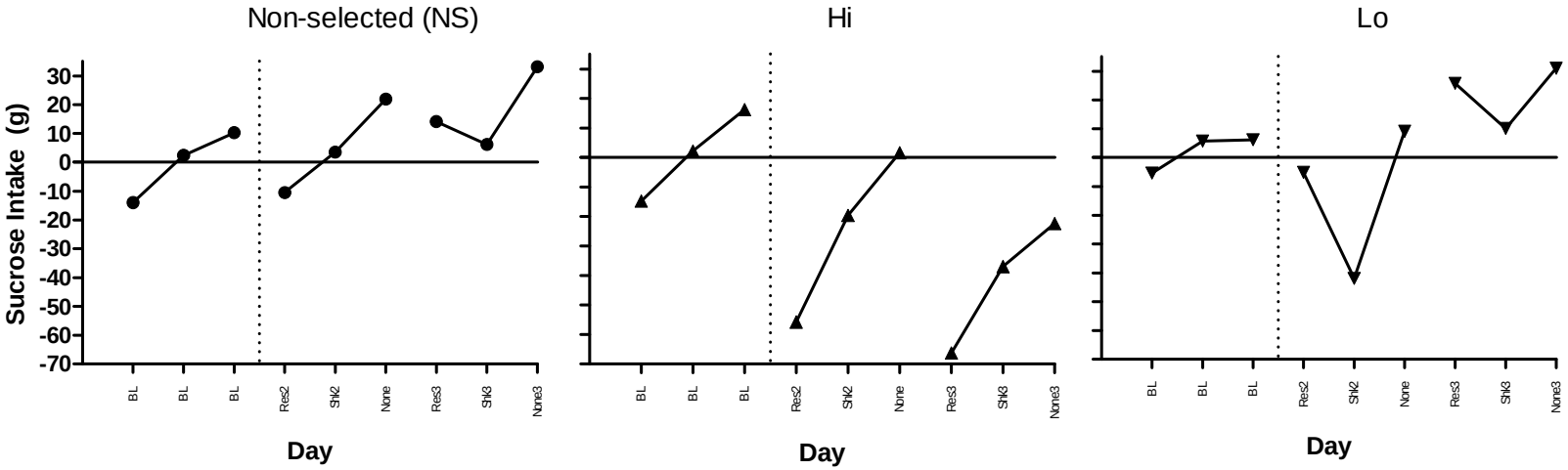


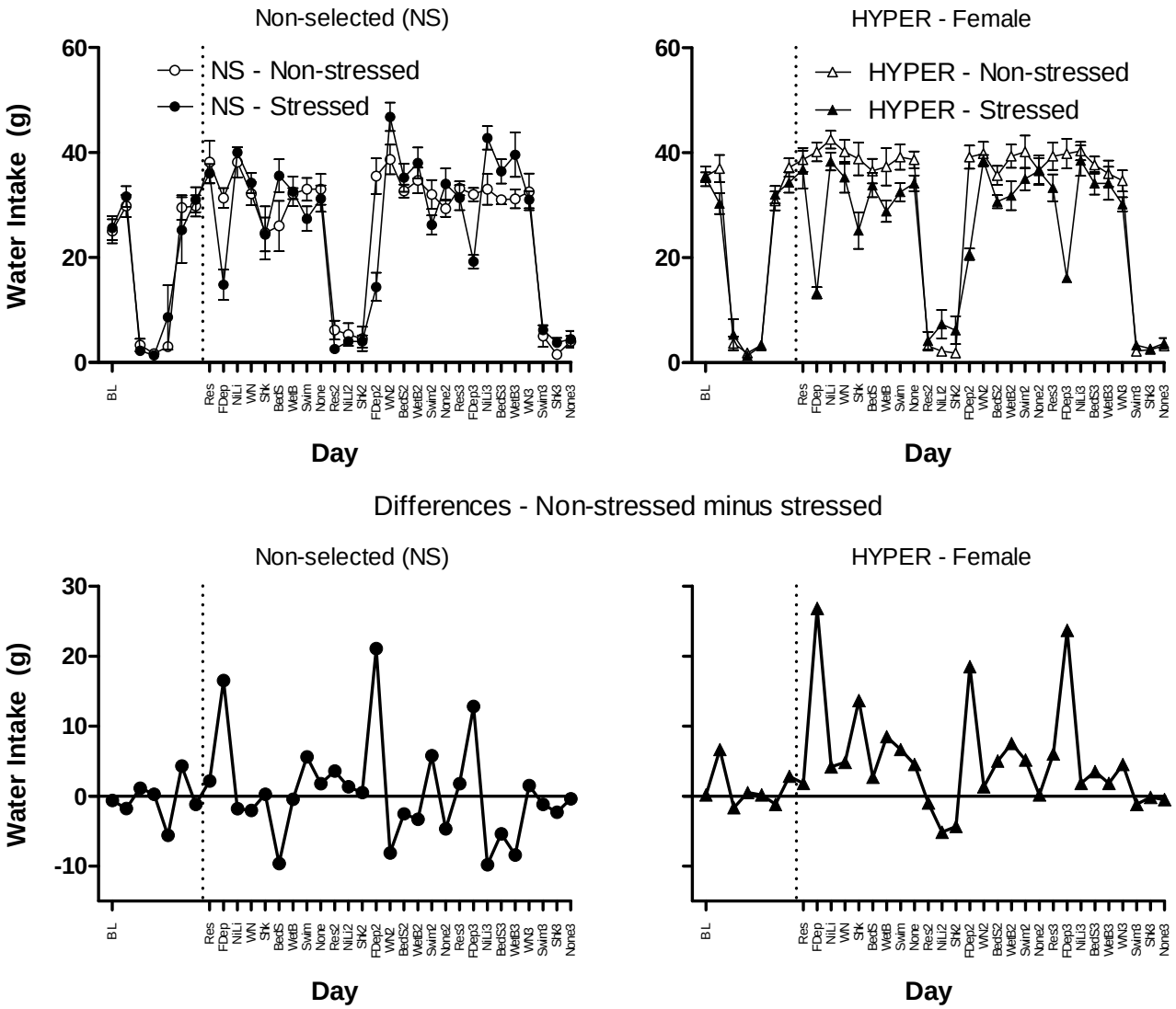
Differences - Stressed minus non-stressed

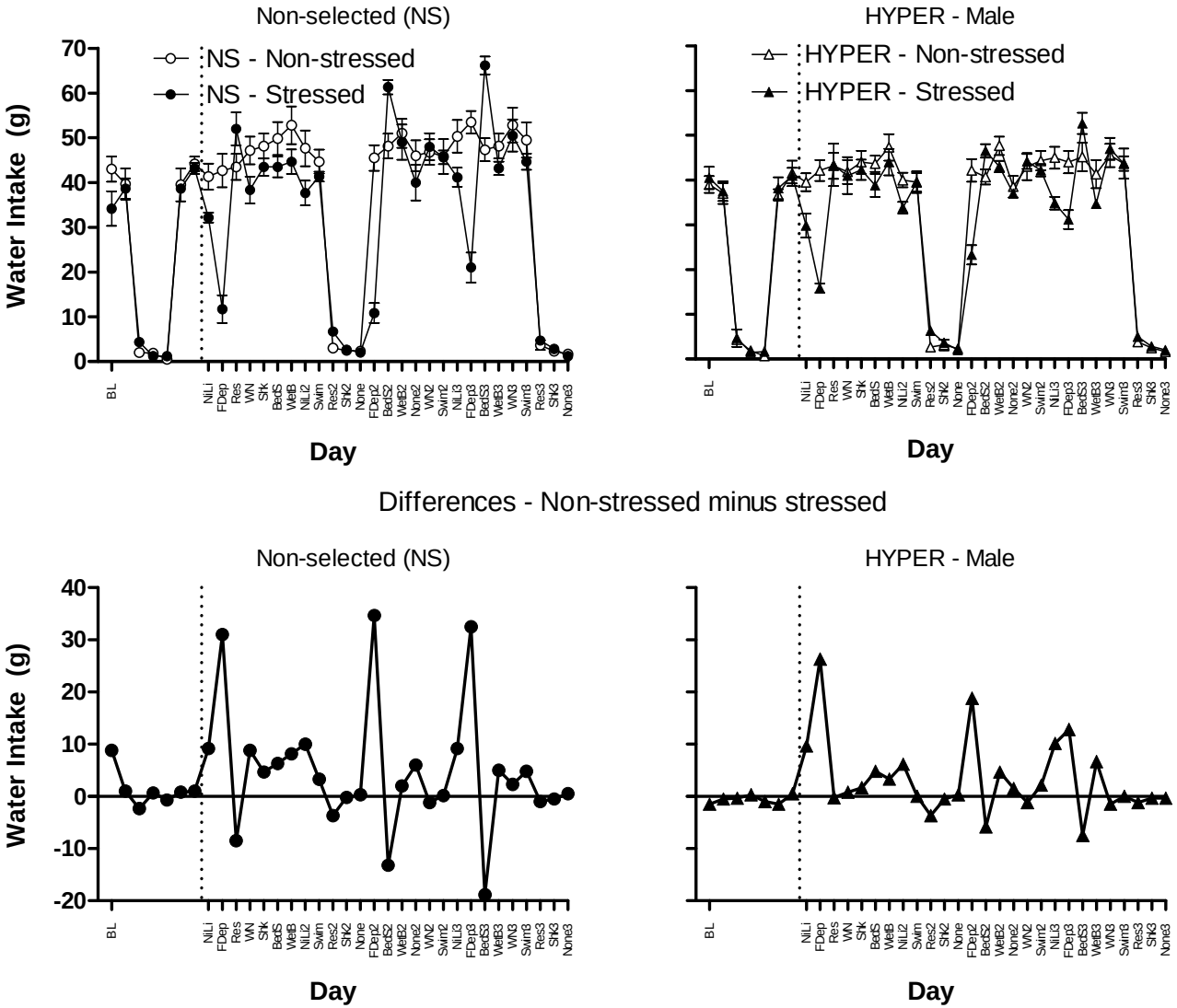


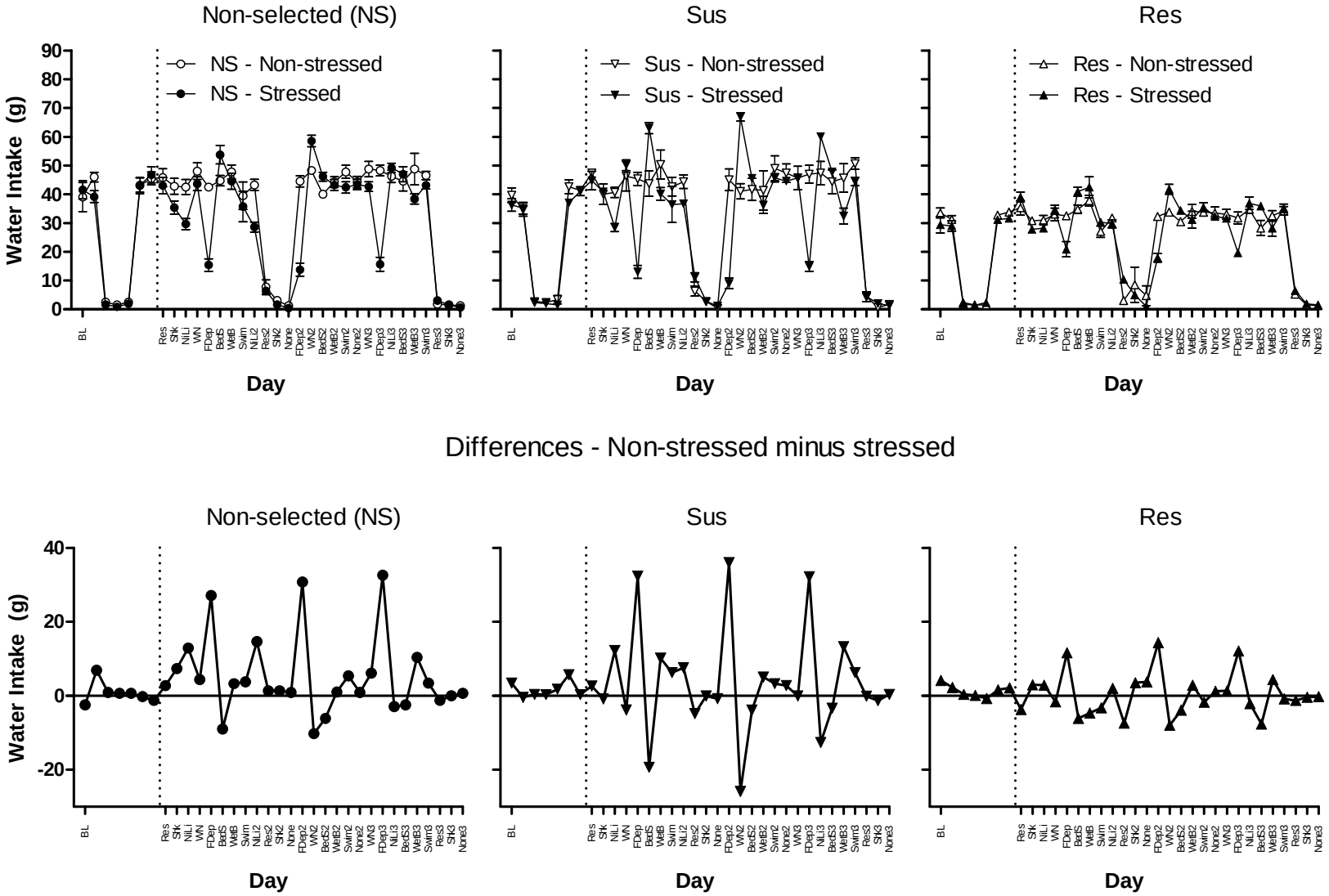


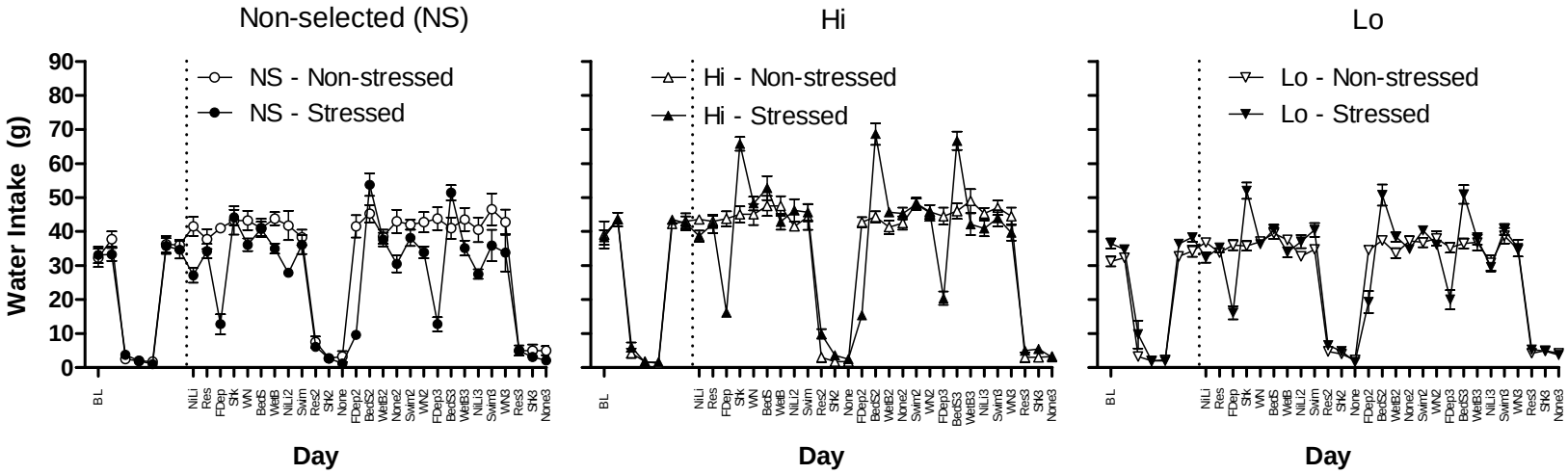
Differences - Stressed minus non-stressed



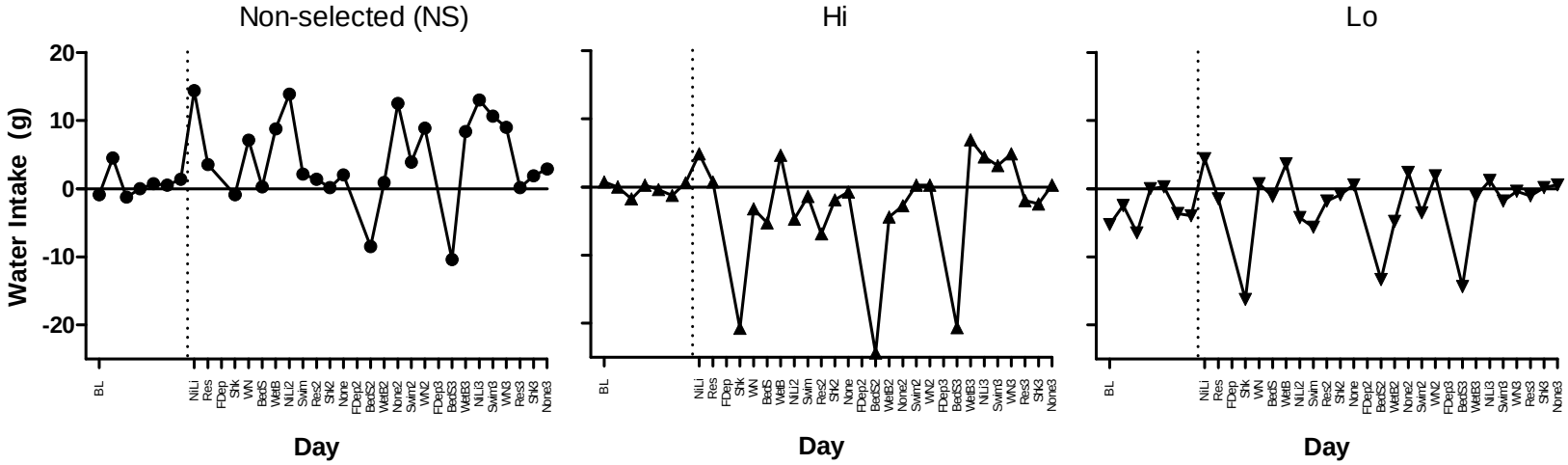

Non-selected (NS)
HYPER - Female
NS - Non-stressed
NS - Stressed
HYPER - Non-stressed
HYPER - Stressed
Water Intake (g)
Day
Differences - Non-stressed minus stressed
Non-selected (NS)
HYPER - Female
Water Intake (g)
Day

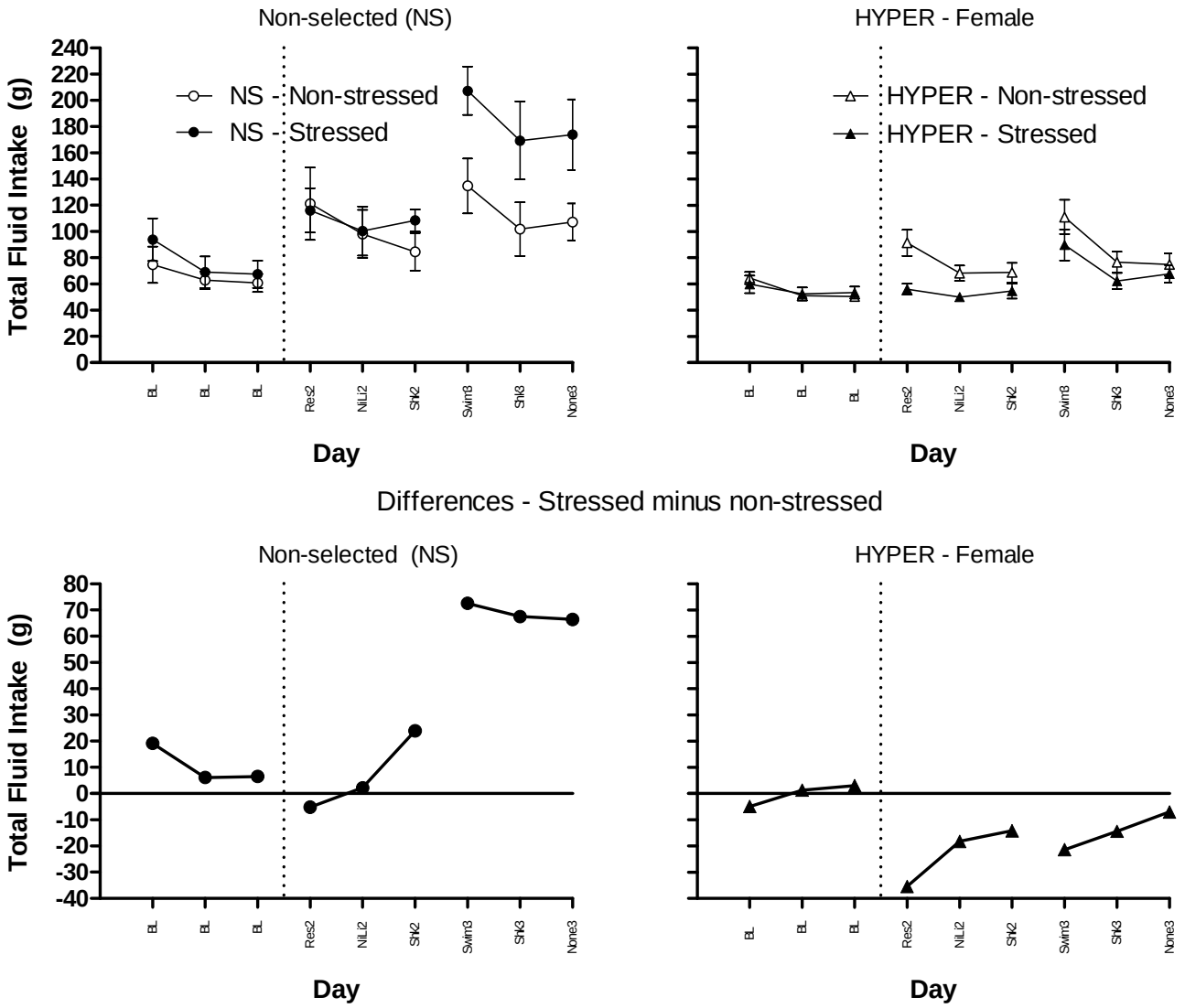

Non-selected (NS)
HYPER - Male
NS - Non-stressed
NS - Stressed
HYPER - Non-stressed
HYPER - Stressed
Water Intake (g)
Day
Differences - Non-stressed minus stressed
Non-selected (NS)
HYPER - Male
Water Intake (g)
Day

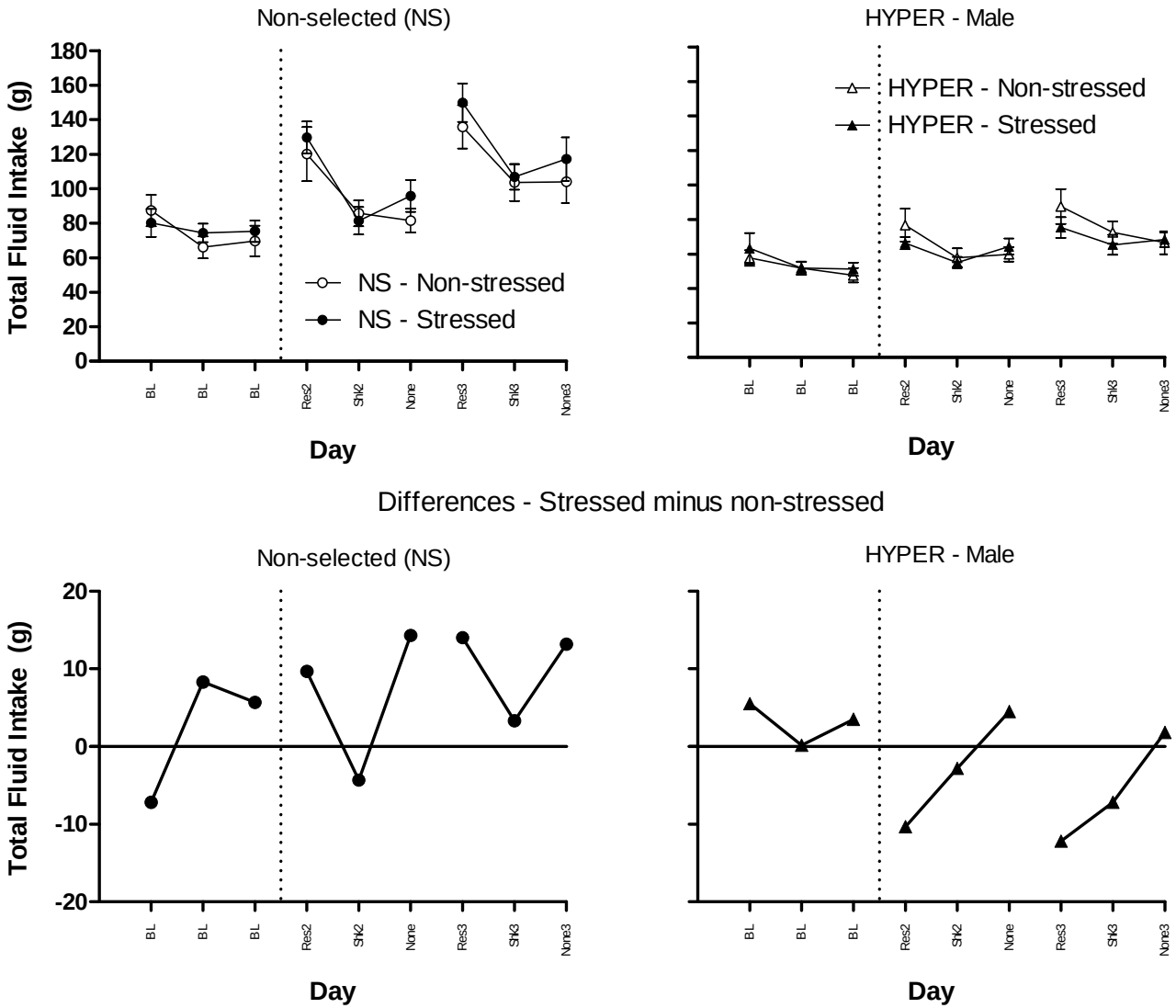

Non-selected (NS)
Sus
Res
NS - Non-stressed
NS - Stressed
Sus - Non-stressed
Sus - Stressed
Res - Non-stressed
Res - Stressed
Water Intake (g)
Day
Differences - Non-stressed minus stressed

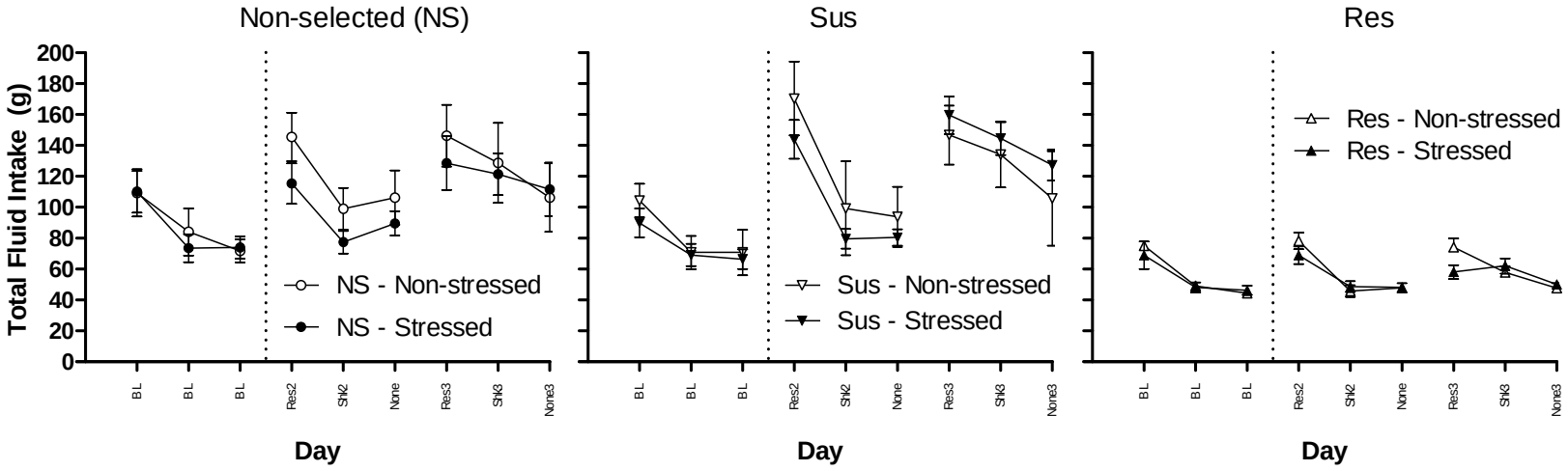


Differences - Non-stressed minus stressed

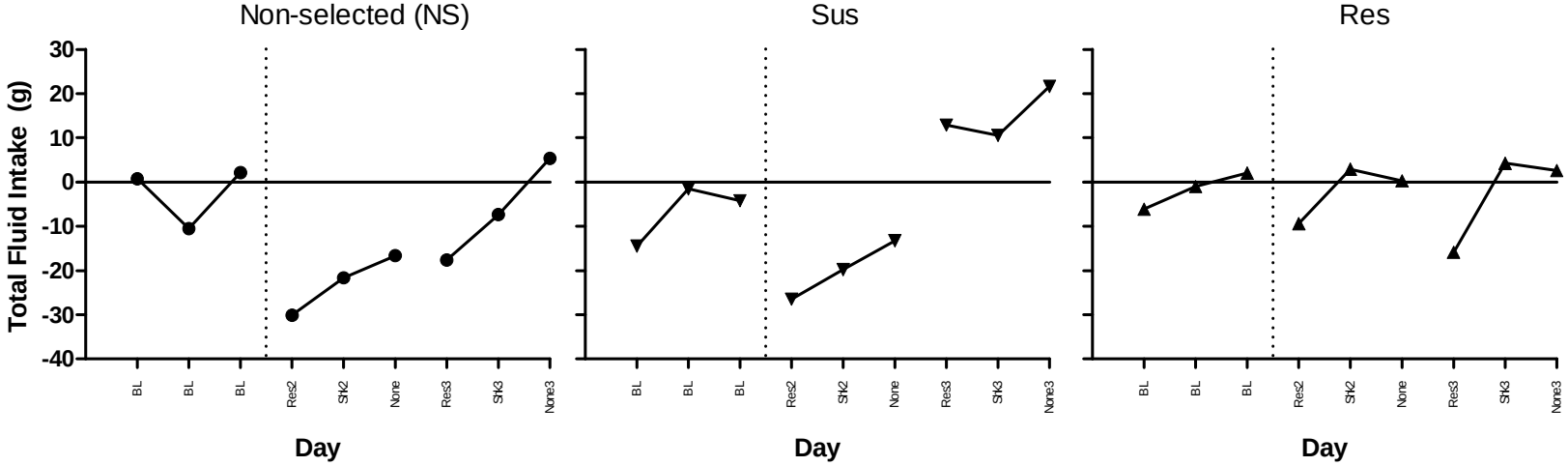


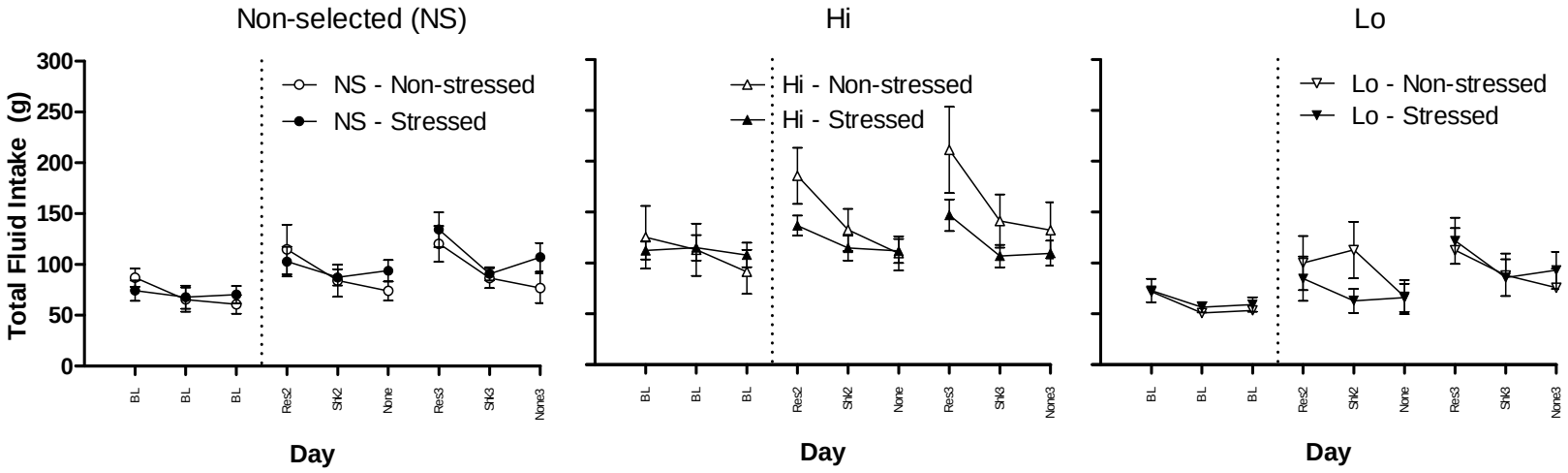

Non-selected (NS)
HYPER - Female
NS - Non-stressed
NS - Stressed
HYPER - Non-stressed
HYPER - Stressed
Total Fluid Intake (g)
Day
BL
Res2
NiLi2
SHK2
Swim3
SHK3
None3
Differences - Stressed minus non-stressed


Non-selected (NS)
HYPER - Male
Total Fluid Intake (g)
180
160
140
120
100
80
60
40
20
0
NS - Non-stressed
NS - Stressed
HYPER - Non-stressed
HYPER - Stressed
BL
BL
BL
Res2
Shk2
None
Res3
Shk3
None3
Day
Differences - Stressed minus non-stressed
20
10
0
-10
-20

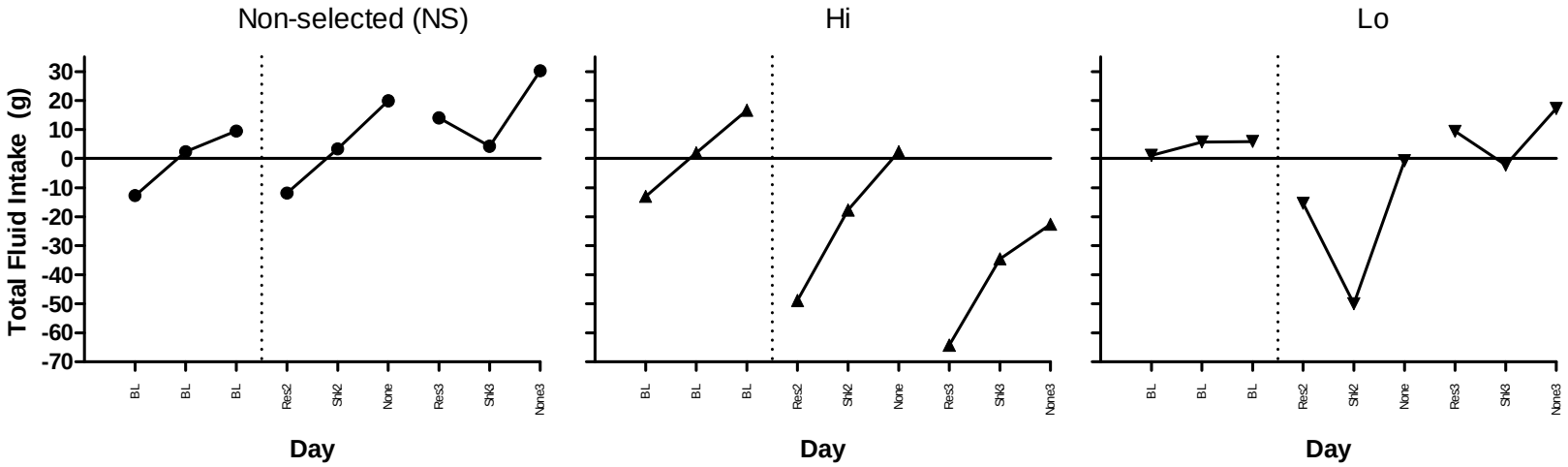


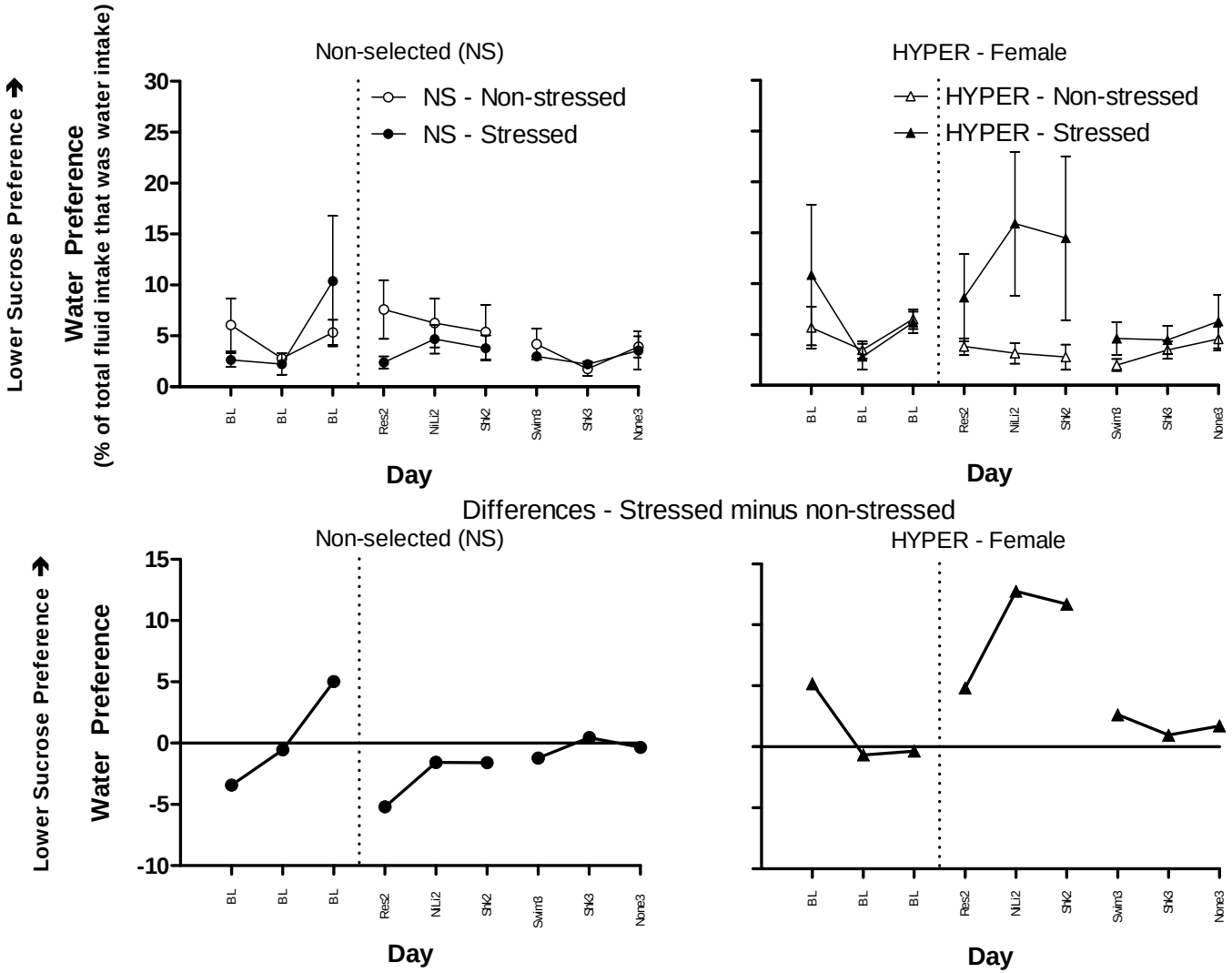
Differences - Stressed minus non-stressed

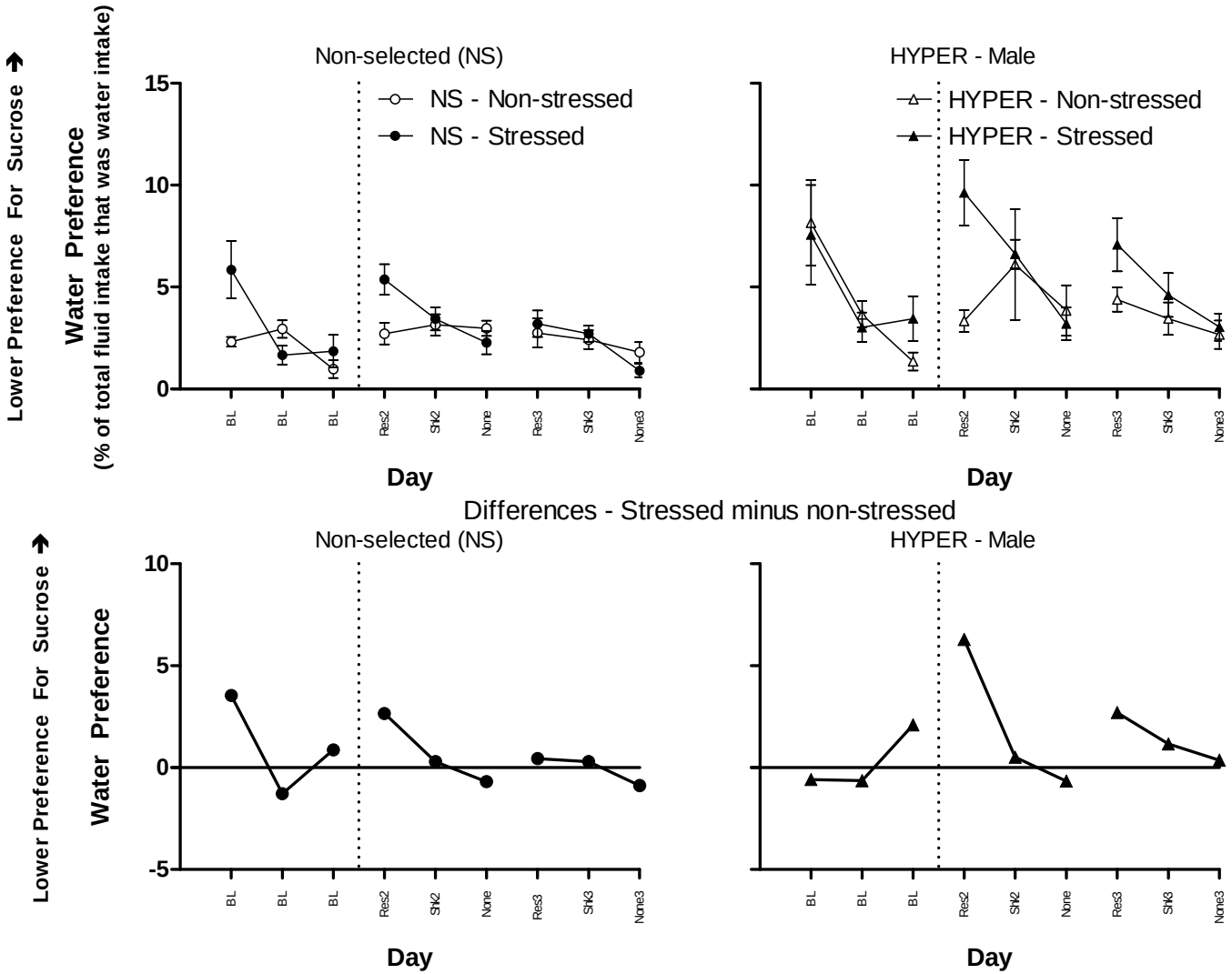


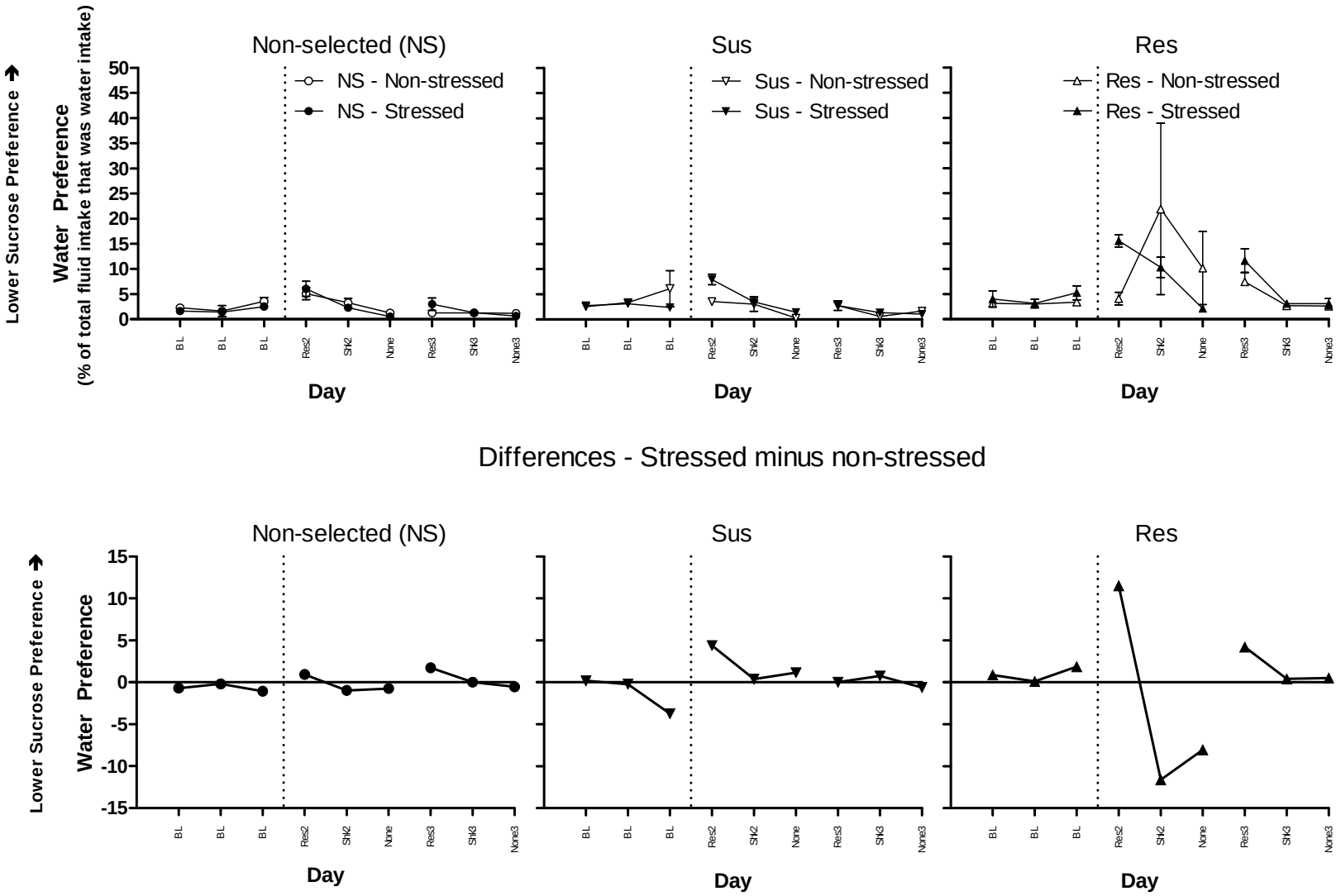


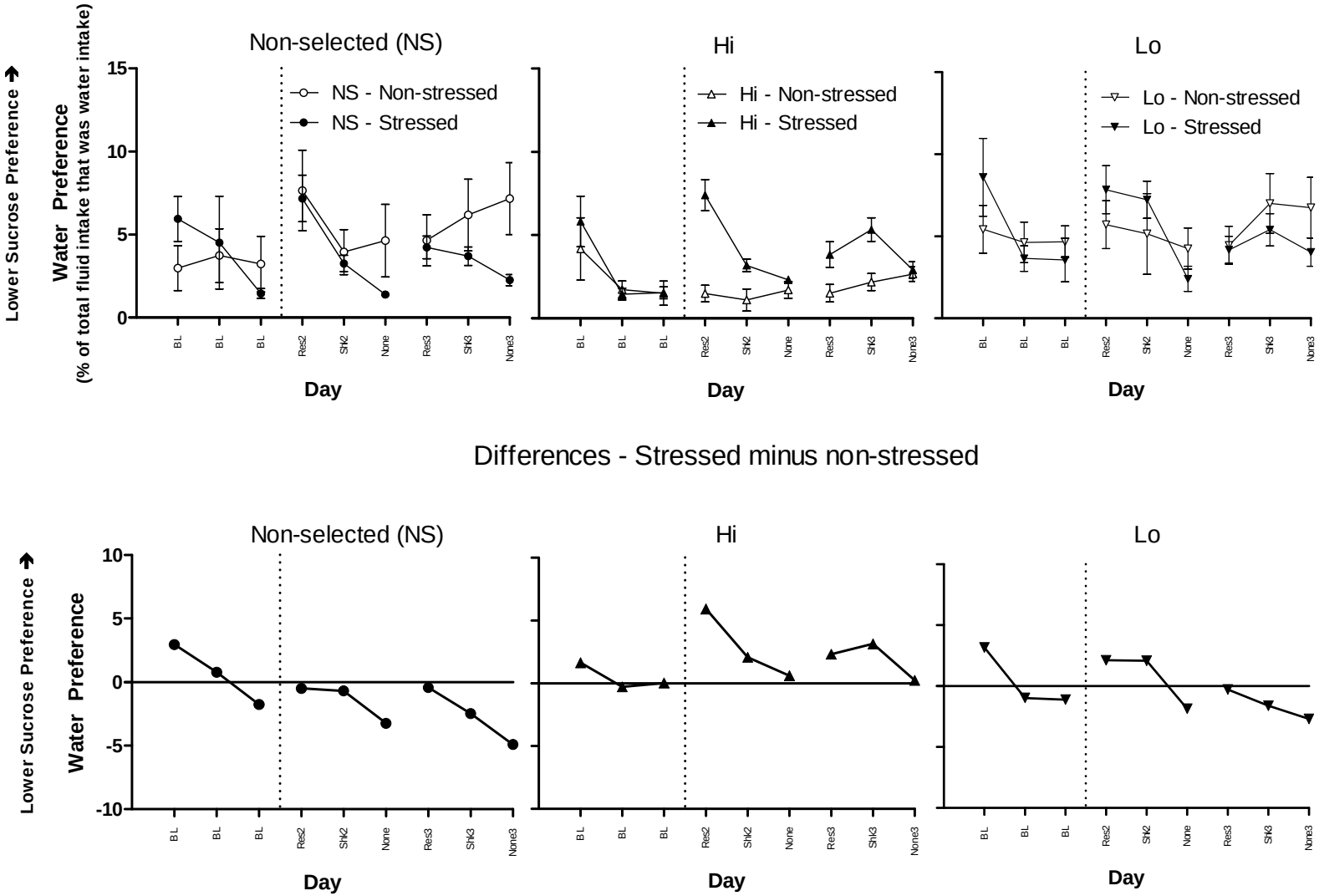
Differences - Stressed minus non-stressed

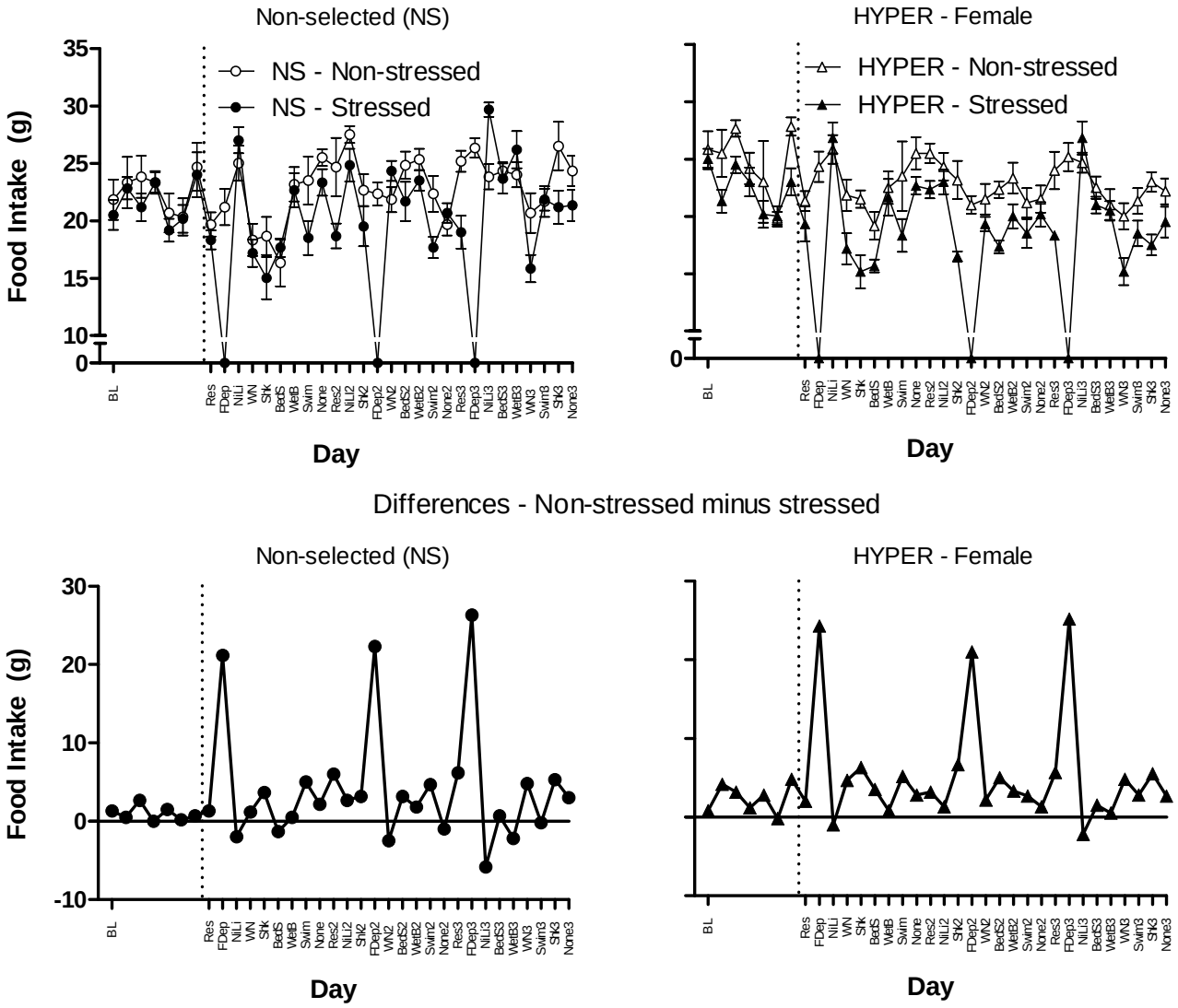


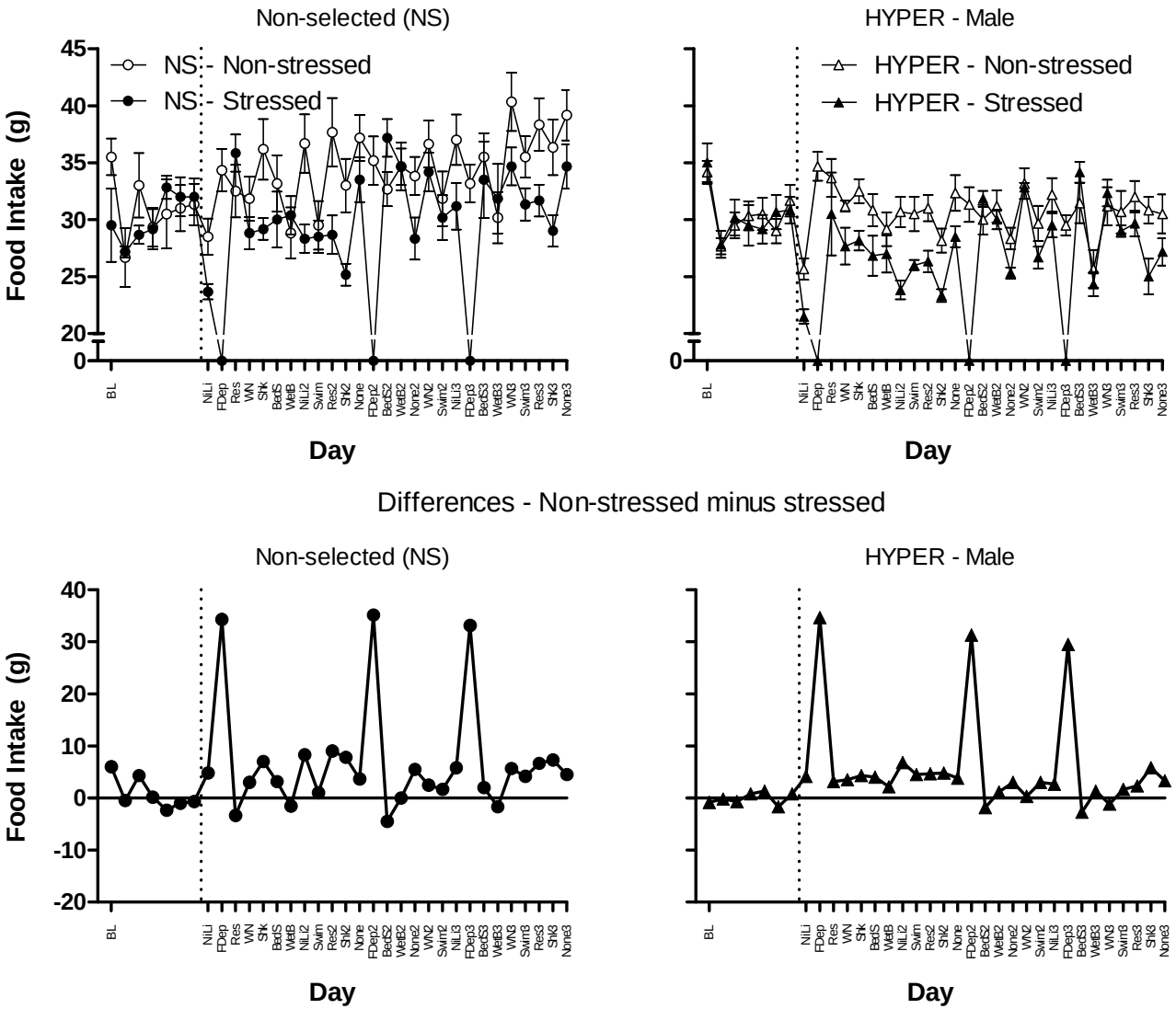

Non-selected (NS)
HYPER - Female
NS - Non-stressed
NS - Stressed
HYPER - Non-stressed
HYPER - Stressed
Lower Sucrose Preference
Water Preference
(% of total fluid intake that was water intake)
Day
Differences - Stressed minus non-stressed
BL
Res2
NiLi2
Shk2
Swim3
Shk3
None3

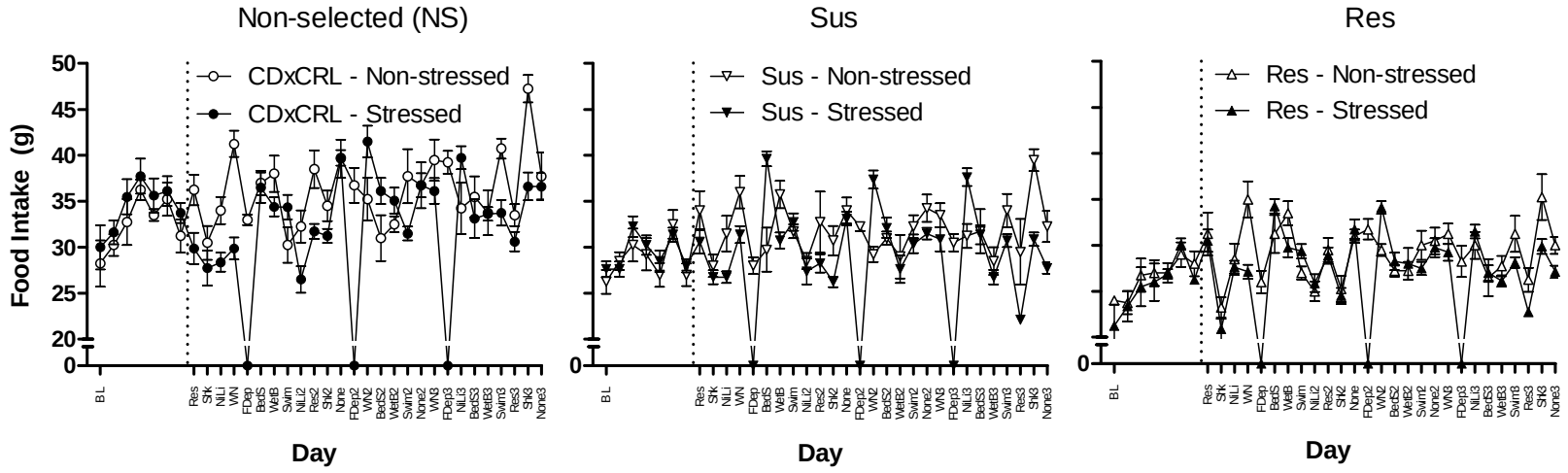

Non-selected (NS)
HYPER - Male
NS - Non-stressed
NS - Stressed
HYPER - Non-stressed
HYPER - Stressed
Lower Preference For Sucrose
Water Preference
(% of total fluid intake that was water intake)
15
10
5
0
BL
BL
BL
Res2
Shk2
None
Res3
Shk3
None3
Day
Differences - Stressed minus non-stressed
Non-selected (NS)
HYPER - Male
Lower Preference For Sucrose
Water Preference
10
5
0
-5
BL
BL
BL
Res2
Shk2
None
Res3
Shk3
None3
Day


Non-selected (NS)
Sus
Res
NS - Non-stressed
NS - Stressed
Sus - Non-stressed
Sus - Stressed
Res - Non-stressed
Res - Stressed
Lower Sucrose Preference
Water Preference
(% of total fluid intake that was water intake)
Day
Differences - Stressed minus non-stressed
B L
Res2
Shk2
None
Res3
Shk3
None3

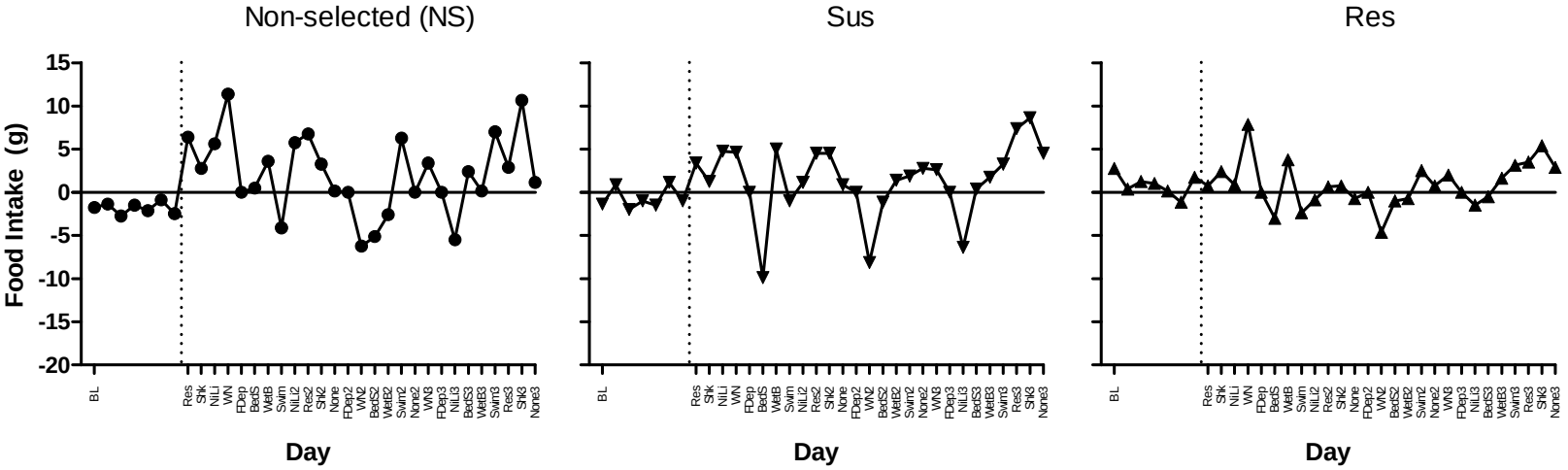

Non-selected (NS)
Hi
Lo
NS - Non-stressed
NS - Stressed
Hi - Non-stressed
Hi - Stressed
Lo - Non-stressed
Lo - Stressed
Lower Sucrose Preference
Water Preference
(% of total fluid intake that was water intake)
Day
BL
Res2
SH2
None
Res3
SH3
None3
Differences - Stressed minus non-stressed

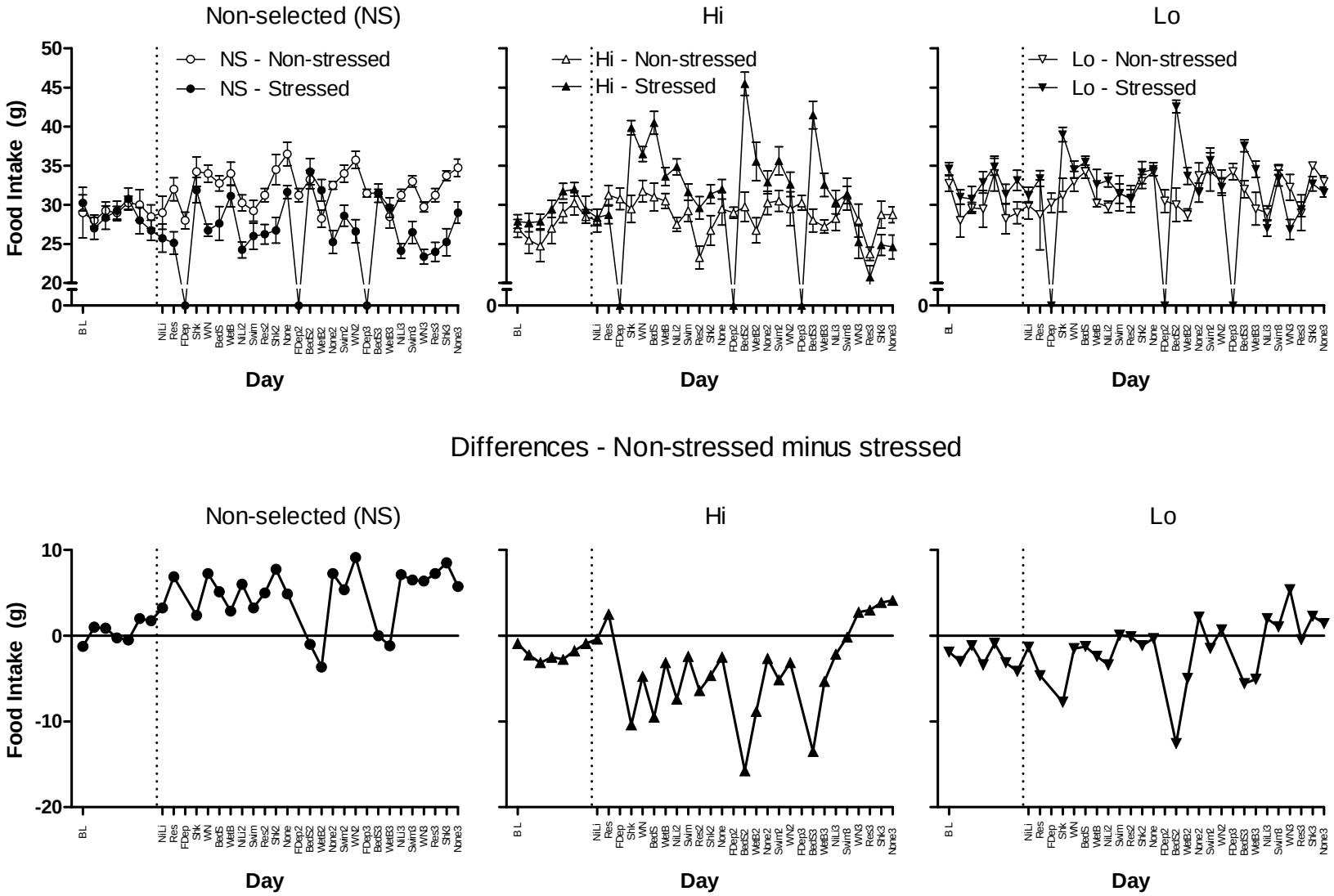

Non-selected (NS)
HYPER - Female
NS - Non-stressed
NS - Stressed
HYPER - Non-stressed
HYPER - Stressed
Food Intake (g)
Day
Differences - Non-stressed minus stressed
Non-selected (NS)
HYPER - Female
Food Intake (g)
Day

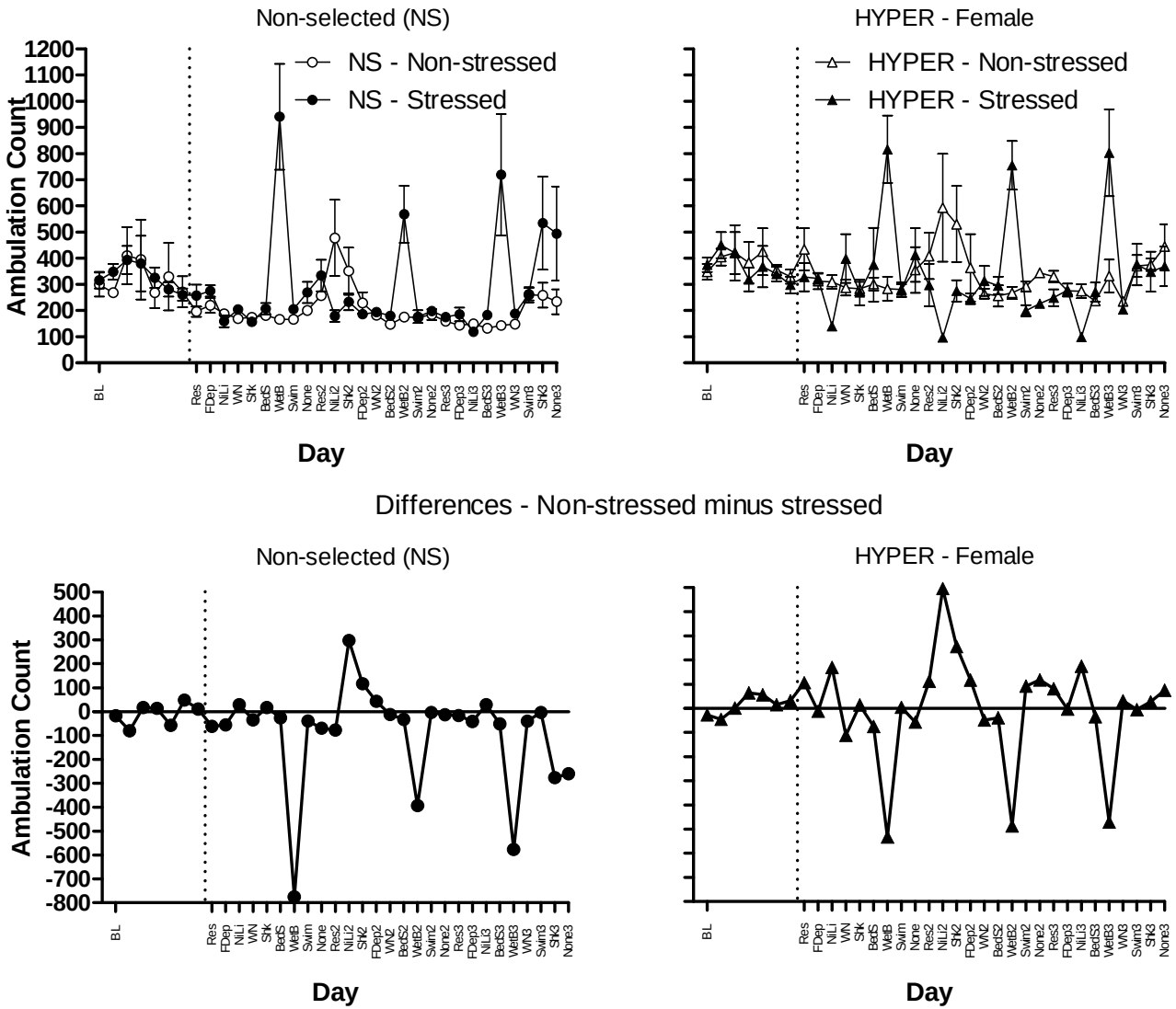

Non-selected (NS)
HYPER - Male
NS - Non-stressed
NS - Stressed
HYPER - Non-stressed
HYPER - Stressed
Food Intake (g)
Day
Differences - Non-stressed minus stressed
Non-selected (NS)
HYPER - Male
Food Intake (g)
Day

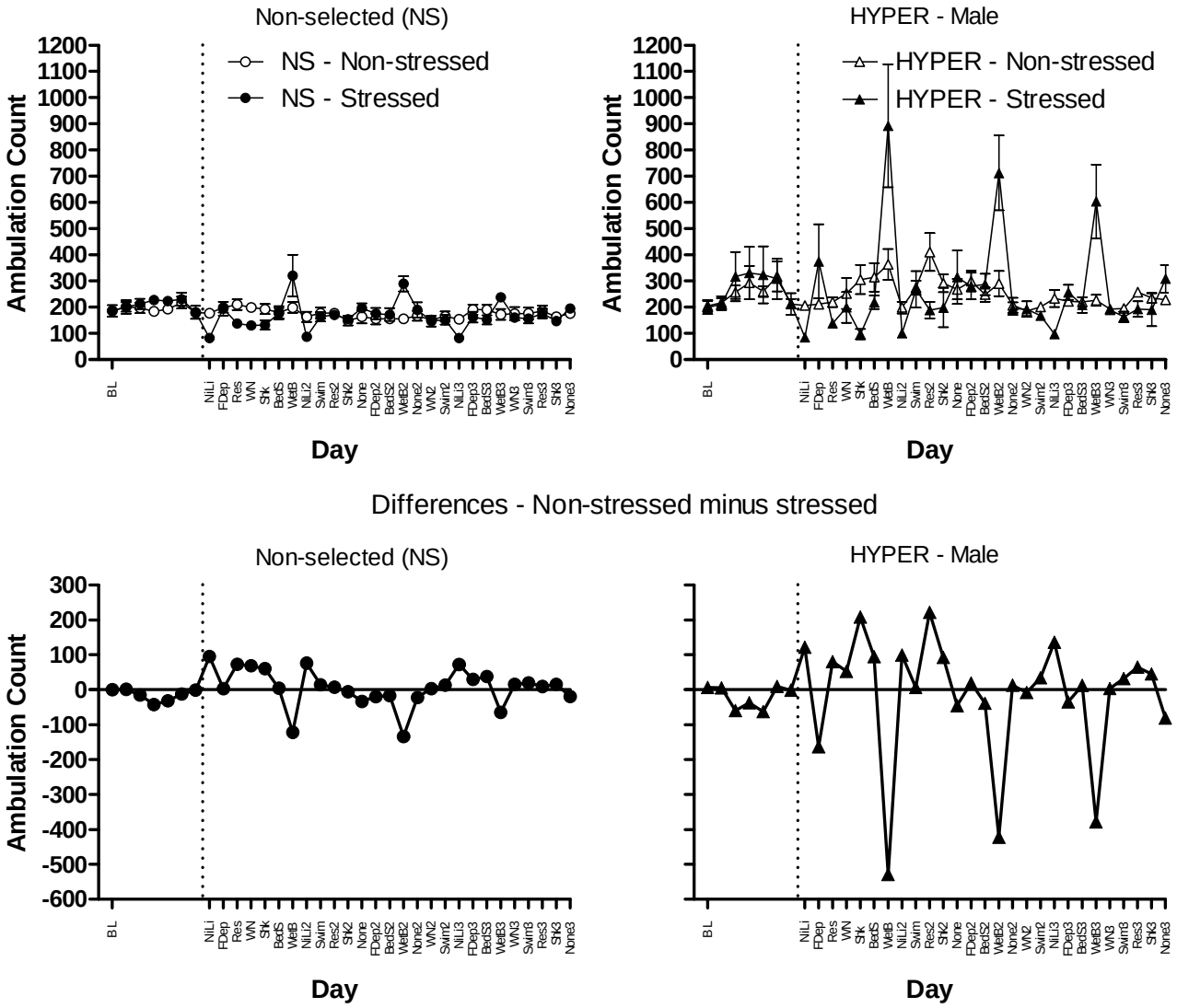


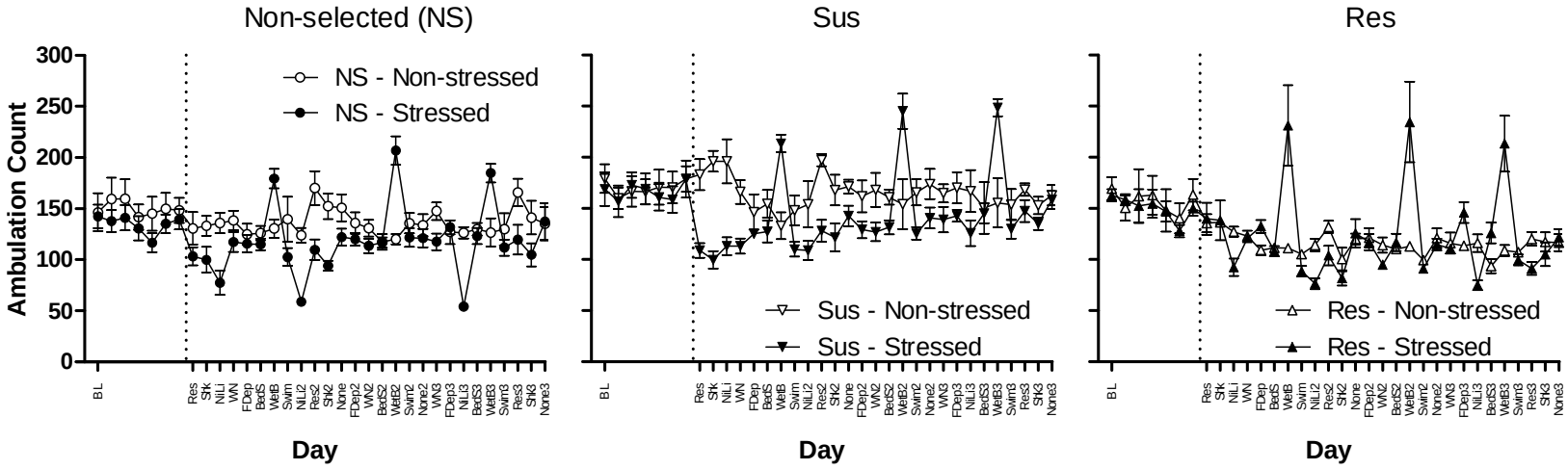
Differences - Non-stressed minus stressed



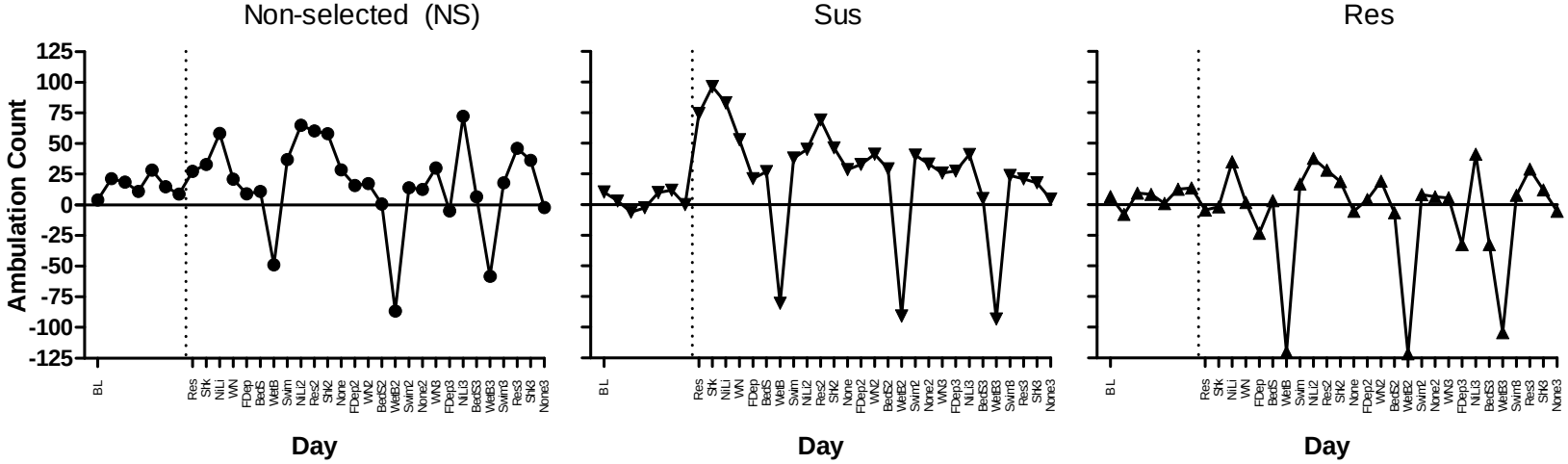

Non-selected (NS)
Hi
Lo
NS - Non-stressed
NS - Stressed
Hi - Non-stressed
Hi - Stressed
Lo - Non-stressed
Lo - Stressed
Food Intake (g)
Day
Differences - Non-stressed minus stressed

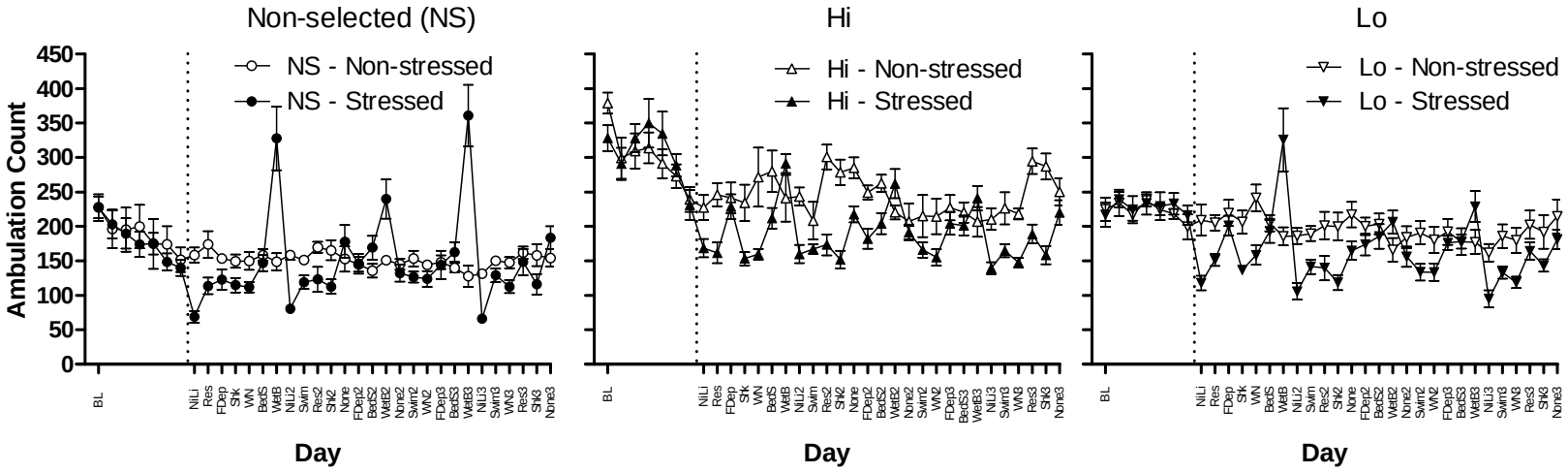

Non-selected (NS)
HYPER - Female
NS - Non-stressed
NS - Stressed
HYPER - Non-stressed
HYPER - Stressed
Ambulation Count
Day
Differences - Non-stressed minus stressed
Non-selected (NS)
HYPER - Female
Ambulation Count
Day


Non-selected (NS)
NS - Non-stressed
NS - Stressed
Ambulation Count
Day
HYPER - Male
HYPER - Non-stressed
HYPER - Stressed
Ambulation Count
Day
Differences - Non-stressed minus stressed
Non-selected (NS)
Ambulation Count
Day
HYPER - Male
Day

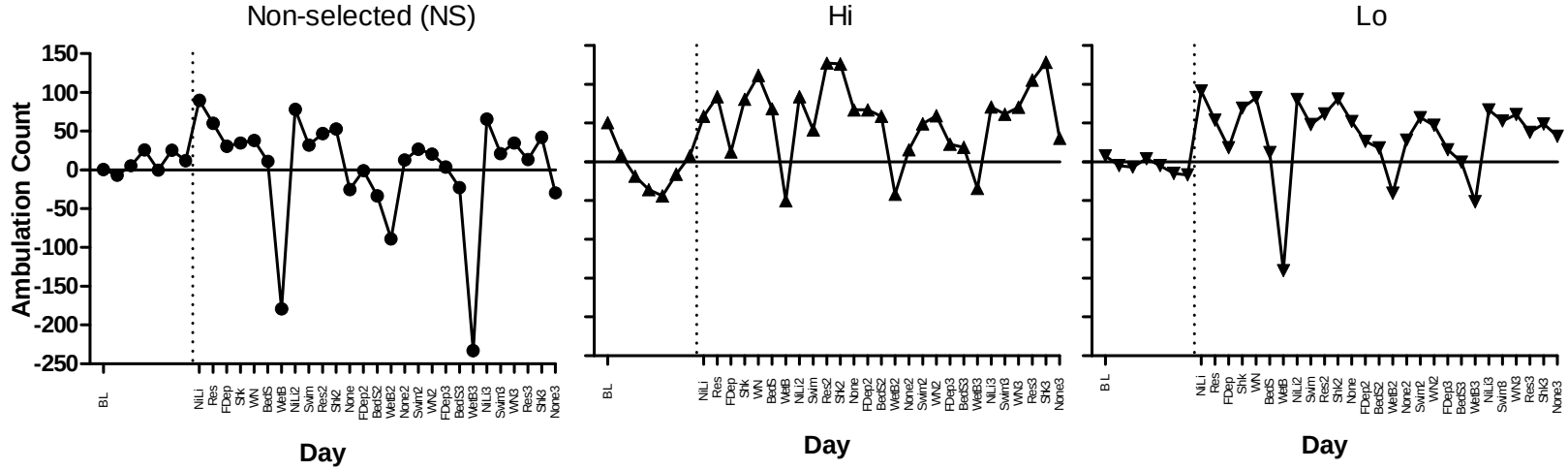


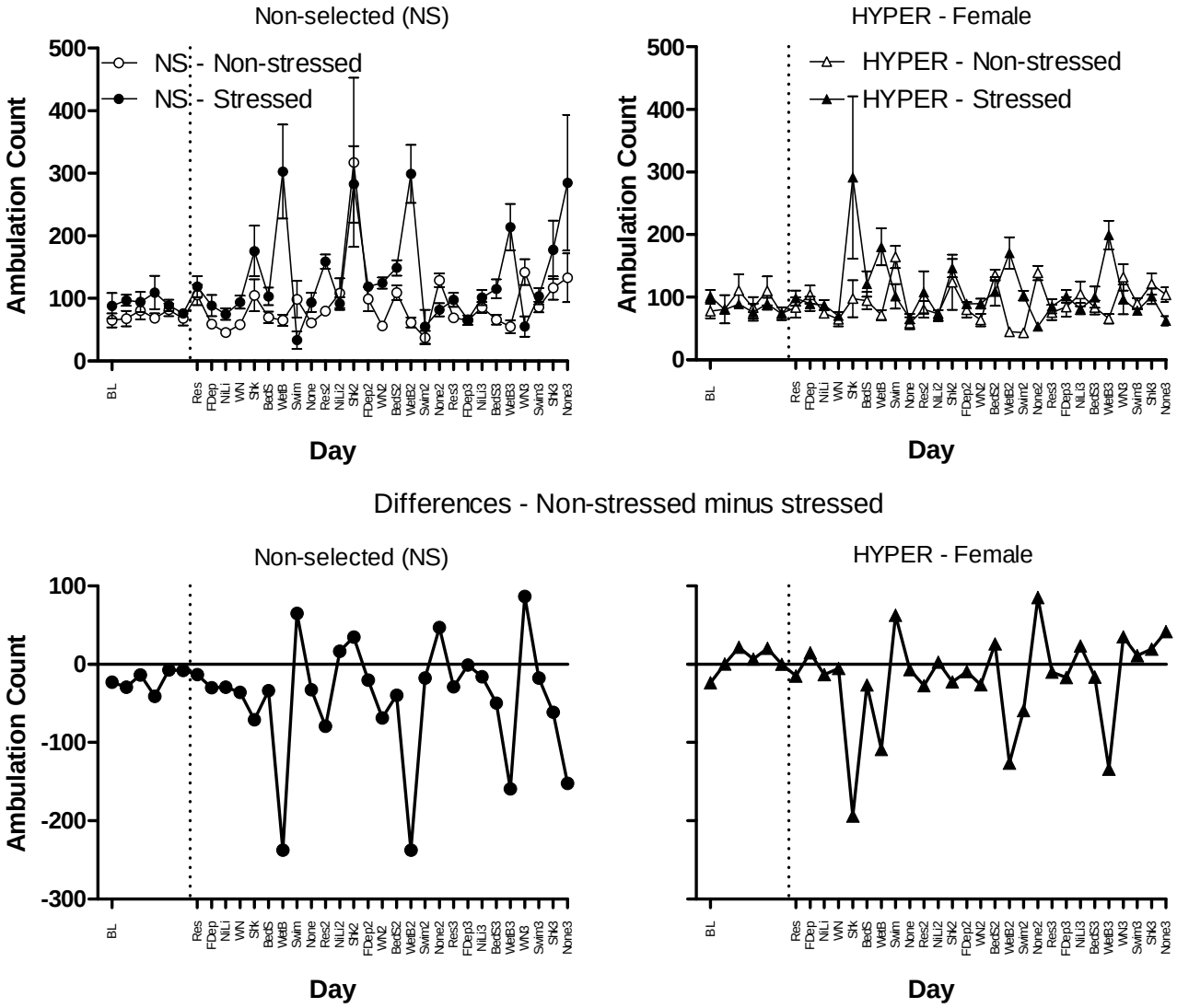
Differences - Non-stressed minus stressed

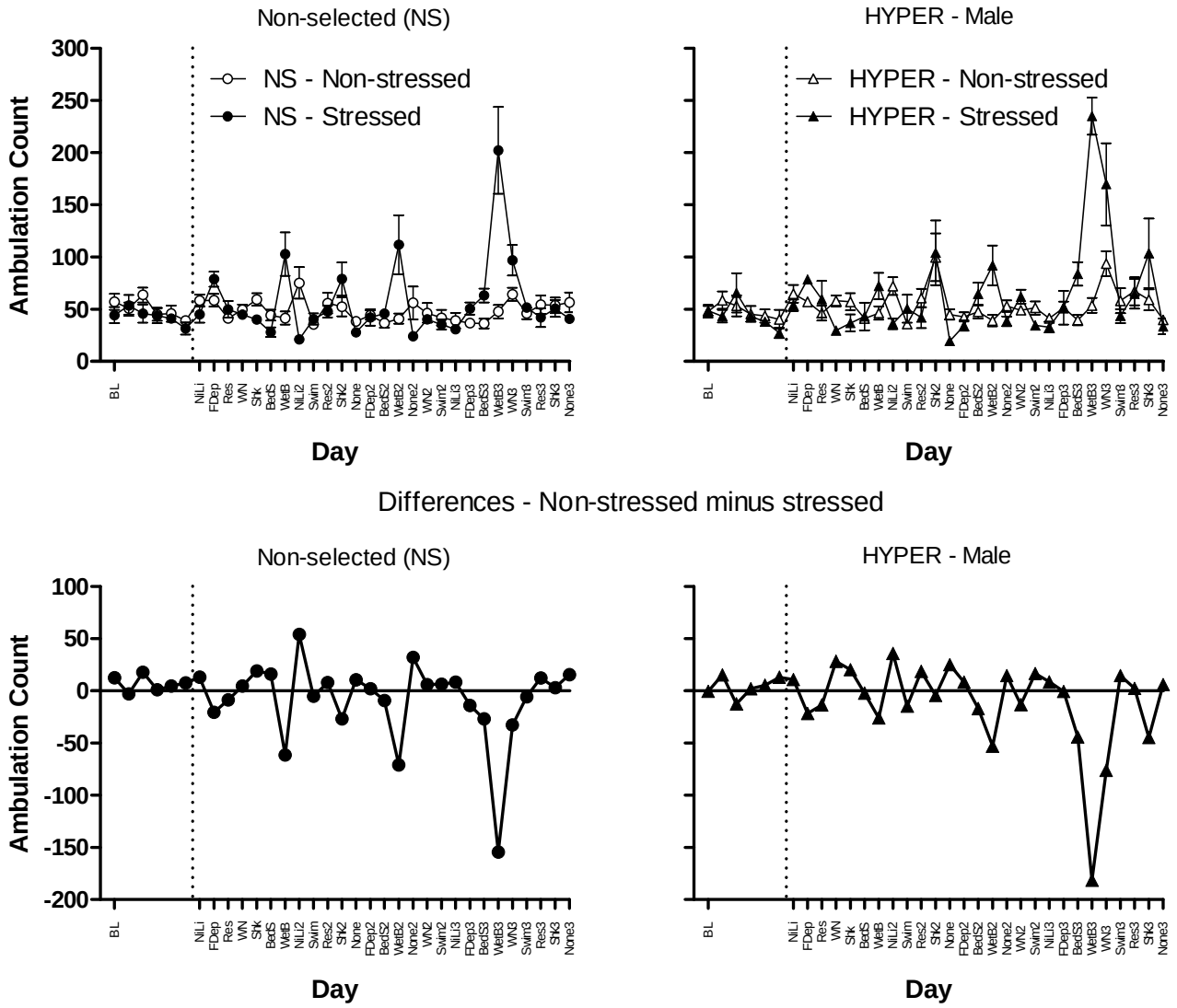


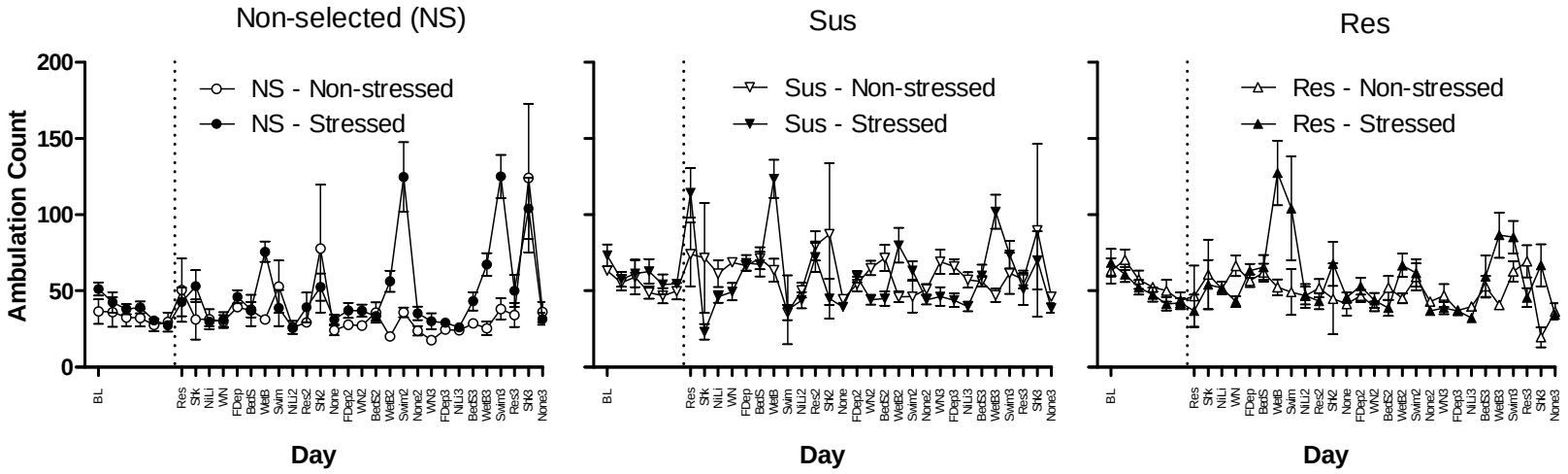


Differences - Non-stressed minus stressed

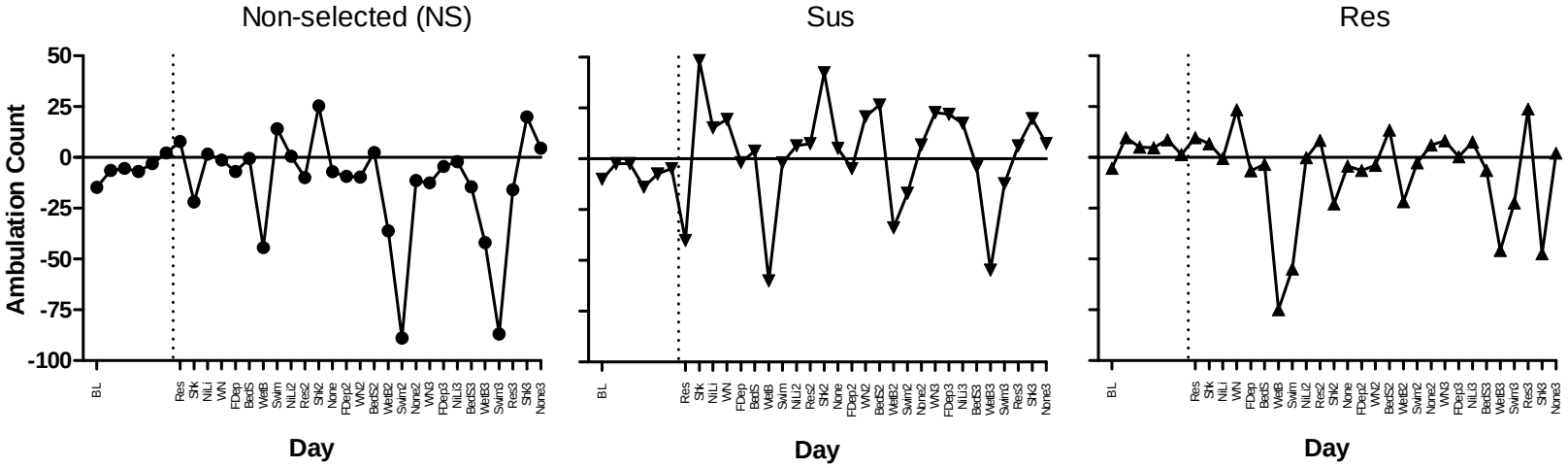


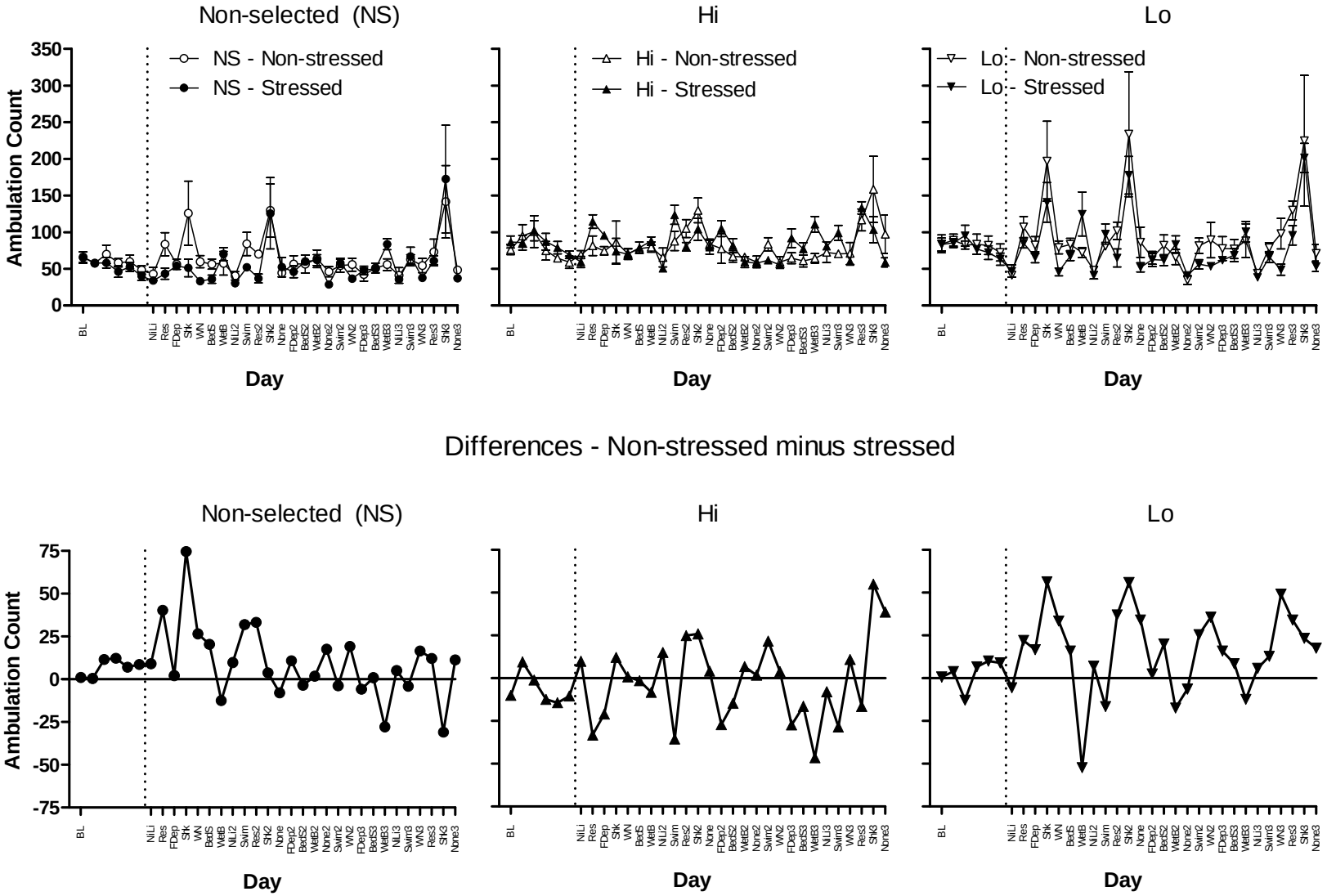

Non-selected (NS)
NS - Non-stressed
NS - Stressed
Ambulation Count
Day
HYPER - Female
HYPER - Non-stressed
HYPER - Stressed
Differences - Non-stressed minus stressed


Non-selected (NS)
HYPER - Male
NS - Non-stressed
NS - Stressed
HYPER - Non-stressed
HYPER - Stressed
Ambulation Count
Day
Differences - Non-stressed minus stressed
B L
NiLi
FDep
Res
WN
Stk
BedS
WetB
NiLi2
Swim
Res2
Stk2
None
FDep2
BedS2
WetB2
None2
WN2
Swim2
NiLi3
FDep3
BedS3
WetB3
WN3
Swim3
Res3
Stk3
None3



Differences - Non-stressed minus stressed




Non-selected (NS)
Hi
Lo
NS - Non-stressed
NS - Stressed
Hi - Non-stressed
Hi - Stressed
Lo - Non-stressed
Lo - Stressed
Ambulation Count
Day
Differences - Non-stressed minus stressed