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Abstract:

Exercise is thought to be beneficial to the body and brain of an individual, but circuitry in the brain involved with executing voluntary exercise is not well understood. Our goal was to evaluate neurochemical differences between mice selected for increased voluntary wheel-running and control mice in order to understand how genetics can influence brain systems involved with locomotor activity, emotive state, learning, and motivation/reward. Wheel-running performance was monitored for eight weeks in selected and control mice derived from an artificial selection experiment for increased voluntary wheel-running. During the last 4.5 weeks, behavior was also evaluated in both lines of mice. At the end of eight week experiment the mice were killed and brain monoamine concentrations were analyzed by HPLC with EC detection. Plasma corticosterone (B) levels were also determined by assay. The neurochemical and hormone differences we report are in mice in a basal condition; they have not had access to running-wheels nor were they stressed. Selected mice ran considerably more than control mice, by means of increased speed and time on the running-wheels. The selected mice were also behaviorally more active during the dark portion of the daily cycle, and devoted very little time to sleep. Levels of B did not differ significantly between the lines of mice, but substantial differences were found in basal monoamine levels. Increased striatal (anterior caudate) DA was found in selected mice, and lower dopaminergic and serotonergic activity was found in DA (SN and VTA) and 5-HT (dRN) cell body regions. Also, lower serotonergic activity was found in regions of the brain associated with emotive state (amygdala), learning (hippocampus), and motivation/reward (nucleus accumbens). The altered DA and 5-HT systems in the selected mice are products of the selection experiment and may help with the understanding of motivation and with disorders such as addiction to drugs, anxiety, depression, and possibly ADHD.

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Introduction:

Exercise tends to elevate mood in humans and is generally good for the body and the brain (Leppimaki *et al*., 2002). Chronic exercise has been used as therapy for treating depression and anxiety disorders possibly through exercise induced alterations in brain monoaminergic activity (Chaouloff, 1989; Dishman, 1997; Fulk *et al*., 2004). In particular, exercise-induced changes in neurological function have been linked to the activity of the monoamines, serotonin (5-HT) and dopamine (DA) (Greenwood, *et al*., 2005; Sutoo and Akiyama, 2003). Interestingly, 5-HT and DA are integral in motivational and reward circuitry in the brain (Phelix and Broderick, 1995; Broderick and Phelix, 1997) and it is possible that voluntary exercise may result in alterations in these circuits because motivation is a factor in exercise. An interesting animal model selecting for increased voluntary exercise was created with house mice (Swallow *et al.*, 1998a). In this model we hypothesize that basal monoamine levels and/or activity in brain regions associated with motivation and reward may be altered within these lines of mice due to genetic differences that predispose some of the mice to engage in vigorous exercise. An understanding of neurological differences in this model may help with our understanding of motivation and with our perception of disorders such as anxiety, depression, and attention deficit hyperactivity disorder [ADHD (see Russell *et al.*, 2005)].

Garland and colleagues artificially selected house mice for increased voluntary wheel-running with the intent to study the evolution of the wheel-running behavior and physiology (Swallow *et al*., 1998a). Physiological capacities were hypothesized to increase concomitantly with activity levels as the selection continued across generations.

However, physiological differentiation between the selected and control lines have not been as pronounced as initially anticipated (Swallow *et al.,* 1998b; Garland *et al.,* 2002; Swallow *et al.,* 2001; Swallow *et al.,* 2005). As a result of selection, changes in the central nervous system, particularly the motivational regions, have been proposed to account for the increased running behavior in the selected lines (Rhodes and Garland, 2003; Rhodes *et al*., 2003a). In addition, "hyperactive" selected mice may be a very good model for attention deficit hyperactivity disorder in humans (Rhodes *et al*., 2005).

Selected mice were running 2.7 times further than the controls after 31 generations of selection (Garland, 2003; unpublished results). The increase in total distance run by the selected mice was accounted for by an increase in running speed (Girard *et al*., 2001, Koteja and Garland, 2001). Kelley *et al.* have recently reported differences in time spent running, but only in males between days 47-52 of their wheel-running study. Additionally, selected mice, when tested in constant darkness vs. constant light, have a shorter (approximately 30 minutes advanced) free-running circadian period (Koteja *et al*., 2003).

Selected mice are generally more active in an accustomed environment (Rhodes *et al*., 2001) and, interestingly, build smaller thermoregulatory nests (Carter *et al*., 2000). However, exposure of selected mice to the novelty of an open field experiment eliminated activity differences with respect to control lines (Bronikowski *et al*., 2001). Additionally, results of previous behavioral experiments, in which photo-beam crossing and instantaneous behavior observation were measured, suggest that selected mice perform activities quicker than control mice (see Rhodes *et al*., 2001; Koteja *et al*., 1999). These ethological differences between the selected and control mice may be elicited, in

part, by neurological variations resulting from artificial selection for increased voluntary wheel-running.

Pharmacological investigations in these mice indicate reduced function of dopaminergic systems in the brains of selected mice (Rhodes *et al*., 2001), possibly due to reduced function of D1-like receptors (Rhodes and Garland, 2003). Furthermore, using Fos-IR to visualize recently activated neurons (Dragunow and Faull, 1989; Zangenehpour and Chaudhuri, 2002), selected mice had changes in the dentate gyrus following wheel withdrawal. These results suggest that the dentate gyrus may be involved in the increased running activity, particularly intensity of running, in selected mice (Rhodes *et al*., 2003a). Consistent with this finding, chronic wheel-running has been shown to increase expression of brain derived neurotrophic factor (BDNF) and neuronal growth in the hippocampus of selected mice compared to the control mice (Rhodes *et al*., 2003b). Curiously, running inhibits learning, which is thought to be linked to the hippocampus, in selected mice when compared to controls. Other brain regions, thought to be involved with motivation for running, including the caudate-putamen, nucleus accumbens, prefrontal cortex, and the lateral hypothalamus exhibited higher expression of C-Fos in selected mice (Rhodes *et al*., 2003a). Some of the differences may be reflected by altered basal monoaminergic activity between the lines of mice; particularly in brain regions associated with motivation, learning, or activity. Changes to serotonergic systems in the brain may also be involved in the increased propensity or heightened motivation of the selected mice to run on wheels. It is also important to study these systems in mice that have not been exposed to running-wheels, to eliminate the wheel effect on the brain.

Although the c-Fos staining experiments suggest the presence of altered neuronal activity between the selected and control mice, potential differences in brain monoamines between the lines are not well understood (Rhodes *et al*., 2005). Therefore, we have examined monoamine concentrations in several systems focusing on 12 brain nuclei throughout the brain and have evaluated the differences between the lines. We are principally interested in the involvement of two general systems in the brain: dopamine and serotonin systems. The mesolimbic DA systems modulate motivation and reward-behavior (Wightman and Robinson, 2002). The nigrostriatal system is important for general locomotor activity (Liste *et al.*, 1997). In addition, dopamine systems are implicated in the stress response. There is also considerable evidence that these systems regulate one another, through reciprocal innervation (Adell and Artigas, 2004, Martin-Ruiz *et al*., 2001). Elevated basal corticosterone (B) concentrations have also been suggested in the selected lines of mice (Girard and Garland, 2002), and altered B levels may influence monoaminergic differences between the lines; therefore, we also evaluate B in this study. Additionally, selected mice perform extreme exercise and they may not respond to stress the same as moderately exercising individuals. Therefore, an evaluation of the B response to stress in these mice may further our understanding of the link between stress and exercise.

Much of the previous research has focused on the effects of withdrawal from exercise in mice selected for increased voluntary wheel-running that have had previous exposure to running wheels. The actual basal neurochemistry of the selected brain regions in these mice has not been studied in depth. An initial examination of the neurochemistry in brain regions associated with reward/motivation under conditions that

have not been confounded by exposure to wheels will elucidate possible differences in monoaminergic function dictated by genetics. The goals of the present research are to: (1) establish general wheel-running behavior for the chosen lines of male mice selected for voluntary exercise (2) ethologically evaluate these mice in an environment different from the one they were selected in (without any wheel history or when the wheel is in the animal's single, exclusive cage) and (3) determine basal monoamine levels in brain nuclei related to exercise, reward, motivation, or learning in these mice. These aims should help to establish a general neurochemical framework in the brain between the control mice and those selected for increased voluntary wheel-running and will allow us to test the following hypotheses: (1) selected mice will have altered DA activity in the nigrostriatal and mesolimbic systems in a basal state allowing them to perform heightened, goal-oriented running behavior, and (2) 5-HT activity in selected mice will also be altered in the amygdala, hippocampus, and nucleus accumbens to explain activity levels, the motivational drive, and reduced learning in these mice. This research should further our understanding of motivation (or addiction) for exercise and may help in the understanding of brain alterations due to diseases such as addictions to drugs and disorders like depression, anxiety, and ADHD.

Materials and Methods:

Animals:

House mice (*Mus domesticus*; Hsd:ICR strain) artificially selected for increased voluntary wheel running were used in this study (Swallow et al., 1998a). Fifty-four male mice [n=26 selected (1 of 4 replicate lines) and n=28 controls (1 of 4 replicate lines)] were obtained from the Garland laboratory (UC-Riverside). Upon arrival at the

University of South Dakota, the mice were housed in groups of 6-8 for approximately two months in Techniplast cages (55 x 35 x 20cm) with wire lids. For the duration of the study, mice were maintained at a constant 23 ^{o}C on a 12:12 reverse light cycle (light from 10 PM-10 AM). Bedding (Harlan Teklad Sani-Chips) was changed weekly at the end of the light period, with food [Harlan Teklad Rodent Diet (W) 8604] and water available *ad libitum*. All procedures were carried out with approval by the University of South Dakota Animal Care and Use Committee and were conducted in accordance with the "Guiding Principles on the Care and Use of Animals" as approved by the Council of the American Physiological Society.

Voluntary Wheel Running:

Mice were reassigned singly to Nalgene cages (47 x 25 x 20cm) equipped with wire lids with (n=24) or without (n=30) an activity wheel (Nalgene F-size wheels) for eight weeks. The activity or running wheel was located within an animal's sole cage and was therefore freely available at all times. This environment is different from the one in which the artificial selection occurred, where a separate, but connected chamber housed the activity wheel (see Swallow et al., 1998a). After the cage reassignment, mice were also weighed during the weekly bedding change (between 9 AM and 10 AM). Twelve selected and 12 control mice had access to the running wheels for the eight week period. The non-wheel group consisted of 14 selected and 16 control animals. Voluntary wheel running was monitored every 60 seconds for the duration of the 8-week experiment via a computer equipped with VitalView software (Mini Mitter Company, Inc., Sunriver, OR). The stainless steel running wheels (1 meter circumference) had a wire bar tread surface and were equipped with a magnetic sensor, allowing detection of distance run (meters),

time spent on wheels (min), and running speed (m/min). Wheel running data are expressed as means per day, unless otherwise noted, across the eight weeks of experimentation.

Behavioral Assessment:

Behavioral observations of all mice were made during the last four weeks of experimentation (days 22-52). Behavior was assessed via instantaneous sampling (Martin and Bateson, 1993) by a single observer (RBP) at three specific times during a 24 hour period starting at 9 AM, 3 PM or 9 PM. During each specific time session, each mouse was scored for ten rounds by instantaneous behavioral scans that were done within 40-45 minutes. During a round, each mouse was scored every 5 seconds. Behaviors were scored in small categories (example: eat, drink, stand/sniff, wheel-run, etc.) and then later lumped into the following mutually exclusive ethological categories: "groom" (licking at fur and rubbing paws), "sleep" (curled up with eyes closed), "idle" (sitting motionless with eyes open), "consumption" (in the motions of eating or drinking), and "active" (moving in cage, running, standing/sniffing, or hanging from wire cage top). Wheel-running is a category that was included in the "active" category for mice with wheel access, but this category was also analyzed separately for comparison with computer generated running data. Also reported is the amount wheel-running behavior in each "active" category for the control and selected mice with wheels. Behavioral data are expressed as frequencies (% time) for each ethological category.

Plasma/Brain Collection:

Following eight weeks of behavioral observations and access to wheels, all mice were killed via spinal dislocation followed by rapid decapitation. Trunk blood was

collected into heparinized tubes, centrifuged, and plasma was stored at -80^{o}C for corticosterone (B) analysis. The extensive results are reported elsewhere (publication in progress). The brain was rapidly dissected from the skull (within 1 min), frozen on dry ice to a microscope slide, and then stored at -80^{o}C until sectioning.

Microdissection and monoamine analysis:

Brains were cut frozen in 300 µm serial sections using a Leica® CM 1800-3 cryostat (Leica Instruments GmbH, Nussloch, Germany) at -11^{o}C. The sections were thaw mounted on glass slides and refrozen for later microdissection. Brain regions were identified using a rat brain microdissection guide (Palkovits and Brownstein, 1988) modified for the size of the mouse brains, and microdissected using a 300 µm id punch. The hippocampus (CA1 and CA3), central nucleus and medial nucleus of the amygdala (CeA and AME), nucleus accumbens (mNacc "core" and lNacc "shell"), anterior caudate (aCPu), paraventricular nucleus (PVN), substantia nigra (SN), ventral tegmental area (VTA), dorsal raphe (DR), and locus coeruleus (LC) were chosen for analysis. These brain nuclei were chosen because they are linked with one or several of the following: exercise, reward, motivation, stress or learning (Dishman, 1997; Chaouloff, 1989; Sutoo and Akiyama, 2003; Wightman and Robinson, 2002). Monoamine analysis of these regions was performed using high performance liquid chromatography (HPLC) with electrochemical detection. Norepinephrine (NE), epinephrine (Epi), dopamine (DA), 3,4-dihydroxy-phenylacetic acid (DOPAC, a metabolite of DA), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA, a metabolite of 5-HT) concentrations were determined for all brain regions (Renner and Luine, 1987). Punched samples were expelled into 60 µl of sodium acetate buffer (pH5) containing 2,3-dihydroxybenzoic acid

(DHBA) as an internal standard, freeze-thawed and centrifuged at 15,000 x g for 5 minutes. Ascorbic acid oxidase (AAO) solution (Sigma Chemical Co., St. Louis, MO, 1 mg AAO/10 ml H_2O) was added (2 µl) to each sample prior to centrifugation. The supernatant was removed and injected (45 µl) into a chromatographic system (Waters Associates, Milford, MA) and analyzed electrochemically with an LC-4B potentiostat (Bioanalytical Systems, West Lafayette, IN). The electrode potential was set at +0.6V with respect to an Ag/AgCl reference electrode. The remaining pellet was solubilized in 110 µl of 0.4 M NaOH and the protein concentration was determined using the Bradford assay (Bradford, 1976). Neurotransmitter concentrations were expressed as pg amine/µg protein. Overall dopaminergic and serotonergic activity was estimated by comparing neurotransmitter and metabolite concentrations.

Statistical analysis:

Wheel-running data were analyzed using analysis of variance (ANOVA, one-way, univariate) between the control and selected individuals. Two-way, univariate ANOVA was used to analyze body mass data. Behavioral assessment generated frequency or percentage data; therefore, an arcsine transformation (Zar, 1999) was applied before running two-way, univariate analysis of variance. Duncan's multiple range tests were used post hoc when significant differences were found with ANOVA. The statistical analyses were done using SPSS 13.0 (SPSS Inc., Chicago, IL) with an alpha of $P<0.05$. Comparing the basal monoamine and corticosterone data between the two lines of mice (control vs. selected) was done using the Student t-test (Sigmaplot 2001, SPSS Inc., Chicago, IL) with an alpha of $P<0.05$.

Results:

Running-wheel Analysis:

Over the eight weeks, mice selected for voluntary wheel-running with access to an activity wheel [12,811 ± 259 (SEM) m/day] ran 3.02 times further than the control mice with wheels (4,247 ± 167 m/day; $F_{1,1294}$=773.39, P<0.001; Fig. 1). In addition, selected mice (23.97 ± 0.364 m/min) ran 1.60 times faster than control mice (14.95 ± 0.339 m/min; $F_{1,1294}$=328.50, P<0.001; Fig. 2), and spent 2.23 times more time (507.27 ± 5.9 min/day) actively engaged in running on the wheels than control mice (227.94 ± 5.84 min/day; $F_{1,1294}$=1132.05, P<0.001; Fig. 3). Selected and control mice spent 35.2% and 15.8%, respectively, of the time available during a day running on their wheels. Patterns of activity within each line did not change significantly across the weeks of the experiment for distance ($F_{7,176}$=0.866, P=0.534), time ($F_{7,176}$=1.391, P=0.212) or speed ($F_{7,176}$=1.188, P=0.312), nor was there a significant interaction between line and week for any variable (distance, $F_{7,176}$=0.672, P=0.696; time, $F_{7,176}$=1.30, P=0.253; speed, $F_{7,176}$=0.233, P=0.977).

Body Mass:

All mice weighed significantly less during week one when compared to later weeks ($F_{8,450}$=5.263, P<0.001; Fig 4). Throughout the eight week experiment, control (38.71 ± 0.174) mice weighed significantly more than selected mice (34.34 ± 0.202; $F_{1,450}$=275.83, P<0.001). Overall, mice with access to a wheel were significantly heavier than those without ($F_{1,450}$=6.032, P=0.014). No significant interactions were found between week and line ($F_{8,450}$=0.262, P=0.978) or between week and wheel ($F_{8,450}$=0.344, P=0.948). However, a significant line by wheel interaction was found such that selected

mice with access to running-wheels (35.03 $\pm$ 0.241) were heavier than those without (33.75 $\pm$ 0.304; $F_{1,450}$=5.919, P=0.015). No interaction was found between week, line and wheel ($F_{8,450}$=0.271. P=0.975).

Behavioral Assessment:

Because mice are nocturnal, the time periods were expected to be different ethologically and, as a consequence, each time was analyzed separately. The last hour of light for the mice was from 9-10 AM. The mid-portion of the dark cycle (when mice are typically most active) was at 3 PM, and 9-10 PM was the last hour of darkness for the animals. Overall behavioral results are illustrated in Fig. 5.

Active Behavior:

At 9 AM, selected mice were more active than controls ($F_{1,752}$=28.93, P<0.001; Fig. 6). However, the presence of the running-wheel did not significantly alter total active behavior ($F_{1,752}$=3.40, P=0.066). A significant line by activity interaction showed that, within the controls, mice with wheel access were more active ($F_{1,752}$=10.29, P=0.001). At 3 PM, selected mice were significantly more active than control mice ($F_{1,752}$=152.26, P<0.001), but overall, wheel and non-wheel groups did not differ ($F_{1,752}$=0.170, P=0.681). There was a trend toward higher activity in control mice with wheel access over those without, as indicated by the line by wheel interaction ($F_{1,752}$=3.672, P=0.056). During the last hour of the dark cycle (9 PM), selected mice showed significantly more active behavior than control mice ($F_{1,698}$=221.63, P<0.001). Additionally, mice with wheel access were more active than those without ($F_{1,698}$=71.47, P<0.001). Higher activity in wheel groups was found within both lines, as indicated by the line by wheel interaction ($F_{1,698}$=10.02, P=0.002).

Consumption Behavior:

During the last hour of light (9 AM) there was no significant effect of either line of mice ($F_{1,752}$=2.97, P=0.085) or wheel access ($F_{1,752}$=0.160, P=0.689) on the amount of time devoted to eating or drinking (Fig. 7). A significant line by wheel interaction was found, indicating control mice with wheels had higher consumption behavior than those without wheels, and selected mice with wheels had a lower frequency of consumption behavior than those without ($F_{1,752}$=3.98, P=0.046). At 3 PM, control mice exhibited higher consumption behavior than selected mice ($F_{1,752}$=6.12, P=0.014), while no differences were found overall between mice with and without a wheel ($F_{1,752}$=0.157, P=0.692). However, a significant line by wheel interaction indicated that control mice with wheels had lower levels of consumption behavior than the non-wheel group, and selected animals with wheels ate and drank more than those without wheels ($F_{1,752}$=46.79, P<0.001). Consumption behavior did not differ between lines at 9 PM ($F_{1,698}$=0.930, P=0.335). Not surprisingly, mice with wheels consumed more than those without wheels ($F_{1,698}$=5.566, P=0.019). A significant line by activity interaction indicates that selected mice with wheels had higher levels of consumption than those without wheels ($F_{1,698}$=7.24, P=0.007).

Idle Behavior:

At 9 AM, there were no significant differences in idle behavior by line ($F_{1,752}$=2.70, P=0.101) or wheel access ($F_{1,752}$=1.19, P=0.274), and there was not a significant interaction between line and wheel ($F_{1,752}$=0.54, P=0.817; Fig. 8). During the middle of the dark phase (3 PM), selected mice ($F_{1,752}$=31.13, P<0.001), as well as mice with wheels ($F_{1,752}$=22.16, P<0.001), were significantly less idle than control and non-

wheel mice, respectively. Within each line, the group with running-wheels showed less idle behavior (line*wheel; $F_{1,752}$=4.26, P=0.039). At 9 PM, control mice were more idle than selected individuals ($F_{1,698}$=13.83, P<0.001). Wheel groups also showed lower levels of idle behavior ($F_{1,698}$=26.09, P<0.001), but no significance was found for the line by wheel interaction ($F_{1,698}$=1.04, P=0.308).

Sleep Behavior:

At 9 AM, control mice slept more than selected mice ($F_{1,752}$=25.68, P<0.001; Fig 9). Also, individuals without wheels were observed sleeping more than those with wheels ($F_{1,752}$=4.09, P=0.043). Additionally, there was a significant line by wheel interaction such that control mice with wheels slept less than control mice without wheels ($F_{1,752}$=5.59, P=0.018). During the middle of the dark period (3 PM), selected mice slept significantly less than control mice ($F_{1,752}$=121.66, P<0.001). No overall differences were observed between wheel and non-wheel groups ($F_{1,752}$=0.007, P=0.933), or within each line ($F_{1,752}$=0.037, P=0.848). During the last hour of the dark cycle (9 PM), selected mice slept significantly less than control mice ($F_{1,698}$=127.41, P<0.001). Also, groups of mice with wheels slept significantly less than non-wheel groups ($F_{1,698}$=38.05, P<0.001), as well as within each line ($F_{1,698}$=6.27, P=0.012).

Grooming Behavior:

At 9 AM, no difference in grooming activity was found between lines ($F_{1,752}$=2.63, P=0.105) or between mice with or without running-wheels ($F_{1,752}$=1.62, P=0.204; Fig. 10). However, the interaction between line and wheel was significant, indicating that selected mice with wheels groomed more than those without wheels, and controls without wheels tended to groom less than those with wheel access ($F_{1,752}$=5.67,

P=0.017). During the middle of the active period (3 PM), selected mice groomed significantly less than controls ($F_{1,752}$=12.28, P<0.001). However, no overall differences were found between wheel and non-wheel groups ($F_{1,752}$=0.909, P=0.341). Within the controls, the wheel group groomed more frequently; in the selected animals, mice with wheels tended to groom less (line*wheel; $F_{1,752}$=10.01, P=0.002). At 9 PM, control mice groomed significantly more than selected mice ($F_{1,698}$=13.25, P<0.001). Additionally, mice with access to wheels groomed less than those without running-wheels ($F_{1,698}$=12.07, P=0.001). Within the selected line, the wheel group exhibited less grooming behavior than the non-wheel category (line*wheel; $F_{1,698}$=11.42, P=0.001).

Wheel-running Behavior:

Selected and control mice used their running-wheels similarly during the 9 AM test period ($F_{1,754}$=0.814, P=0.367; Fig. 11). However, at 3 PM ($F_{1,754}$=48.27, P<0.001) and 9 PM ($F_{1,700}$=64.61, P<0.001), selected mice were observed running significantly more than control mice. At 9 AM, 62% of the "active" behavior observed in control mice consisted of wheel-running. For selected mice, 53.3% of their "active" behavior was wheel-running. During the middle of the dark phase (3 PM), 49.1% and 79.9% of the "active" behavior was running on the wheels for control and selected animals, respectively. At the end of the dark phase (9 PM), the amount of wheel-running behavior within the "active" category was 46.9% for control mice and 68.7% for the selected mice. Based on the behavioral observations, selected mice spent 1.4 times more time on the running-wheels than the control mice (controls, 51% "active" time running on wheels; selected, 71.2% "active" time using the running-wheels).

Plasma Coricosterone:

Basal corticosterone levels did not differ between the selected and control mice (t=0.627, P=0.541; Fig. 12). Additional corticosterone results are reported elsewhere (publication in progress), but a trend was found toward elevated B levels in selected mice, which supports data generated by Garland *et al*. at UCR (data and figures not shown).

Basal Monoamines:

These mice have not had wheel access and were not subjected to stress.

NE:

The basal concentration of NE was significantly higher in selected mice over control mice in the paraventricular nucleus (t=2.68, P=0.019; Fig. 13). The amount of NE was significantly lower for selected mice compared to controls in the medial n. accumbens (t=2.15, P=0.051) and dorsal raphe (t=3.26, P=0.006). Additionally, a trend toward lower NE was found in selected mice compared to control mice in the ventral tegmental area (t=1.89, P=0.081), the CA_3 region of the hippocampus (t=1.95, P=0.073), and the central n. of the amygdala (t=1.90, P=0.081). No significant differences in basal NE were found between lines for the substantia nigra, anterior caudate, lateral nucleus accumbens, locus ceruleus, CA_1 of hippocampus, or the medial amygdala (P>0.10).

Epi:

The concentration of Epi was significantly higher in selected mice compared to control mice in the LC (t=3.40, P=0.005; Fig. 14). Additionally, Epi concentrations were lower in the medial amygdala of mice selected for voluntary wheel-running when compared to control mice (t=2.11, P=0.051). However no significant differences in Epi

were found between the lines in the following brain regions: substantia nigra, anterior caudate, paraventricular nucleus, ventral tegmental area, lateral and medial nucleus accumbens, dorsal raphe, CA_1 and CA_3 regions of the hippocampus, and the central nucleus of the amygdala ($P>0.10$).

DOPAC:

Concentrations of the DA metabolite, DOPAC, were lower in selected mice compared to control mice in the substantia nigra ($t=3.95$, $P=0.002$), ventral tegmental area ($t=3.89$, $P=0.002$), and the dorsal raphe ($t=2.72$, $P=0.018$; Fig. 15). No significant differences in DOPAC were found between control and selected mice in the following brain nuclei: anterior caudate, paraventricular nucleus, lateral and medial accumbens, locus ceruleus, CA_1 and CA_3 regions of the hippocampus, and the central and medial nuclei of the amygdala ($P>0.10$).

DA:

The concentration of DA was significantly higher in selected mice compared to control mice in the anterior caudate ($t=2.65$, $P=0.02$; Fig. 16). Also, DA concentrations were lower for selected mice over controls in the ventral tegmental area ($t=3.00$, $P=0.01$) and the dorsal raphe ($t=3.96$, $P=0.002$), with a similar trend also found in the substantia nigra ($t=2.02$, $P=0.064$). No significant differences in basal DA were found between the lines of mice in the following regions: paraventricular nucleus, lateral and medial accumbens, locus ceruleus, CA_1 and CA_3 regions of the hippocampus, and central and medial nuclei of the amygdala ($P>0.10$).

DOPAC/DA:

There was a trend toward a lower ratio of DOPAC/DA in selected mice compared to control mice in the substantia nigra ($t=2.07$, $P=0.059$; Fig. 17). No significant difference in DOPAC/DA was found in all other brain regions reported in the previous DOPAC and DA sections ($P>0.10$).

5-HIAA:

The concentration of the 5-HT metabolite, 5-HIAA, was significantly lower in selected mice compared to control mice in the all of the following brain regions: substantia nigra ($t=3.00$, $P=0.01$), ventral tegmental area ($t=3.28$, $P=0.006$), lateral accumbens ($t=2.20$, $P=0.05$), medial accumbens ($t=4.65$, $P<0.001$), dorsal raphe ($t=3.20$, $P=0.007$), CA_1 of hippocampus ($t=2.17$, $P=0.049$), CA_3 of hippocampus ($t=2.23$, $P=0.044$), and the medial amygdala ($t=2.62$, $P=0.021$; Fig. 18). However, no significant differences were found in the anterior caudate, paraventricular nucleus, locus ceruleus, or the central nucleus of the amygdala ($P>0.10$).

5-HT:

Concentrations of serotonin were lower in selected mice compared to controls in the anterior caudate ($t=3.38$, $P=0.005$), dorsal raphe ($t=2.15$, $P=0.05$), and the medial amygdala ($t=2.30$, $P=0.039$; Fig. 19). No significant differences were found in 5-HT concentrations between the lines in the following brain nuclei: substantia nigra, paraventricular nucleus, ventral tegmental area, lateral and medial accumbens, locus ceruleus, CA_1 and CA_3 regions of the hippocampus, and central and medial amygdala ($P>0.10$).

5-HIAA/5-HT:

The ratio of 5-HIAA/5-HT was significantly lower in selected mice compared to control mice in the locus ceruleus ($t=3.90$, $P=0.002$; Fig. 20). No significant differences in the ratio of metabolite to transmitter were found in all other brain regions reported in the previous 5-HIAA and 5-HT sections ($P>0.10$).

Discussion:

Wheel-running/Body Mass/Behavior:

The results of the wheel-running component of the experiment indicate that selected mice run three times further than the control mice in a given 24 hour period (Fig. 1). The increased distance run by selected mice was accomplished not only by an increase in the speed of running, but also by the mice spending significantly more time on the wheels (Figs. 2 and 3). Selected mice also weighed less than the controls, and mice having access to wheels were heavier than non-wheel mice, in general. However, only within the selected line were mice with wheel access significantly heavier than the non-wheel group.

The distance these male selected mice ran is very comparable to previous monitoring of the selected and control lines in other laboratories (Swallow *et al.*, 1998a; Garland, 2003; unpublished results). Like these other reports, we also found that the selected line of mice ran faster than the controls, but unlike these other studies, we found selected mice spent much more time on the running-wheels than the control mice. This may be explained by the different environment that was used in our study. Wheel-mice in our experiment were housed in a cage in which the wheel was directly accessible, allowing them to run on a whim. In the other experiments, the running-wheel was

located in a separate chamber, accessed via a tunnel, from where the mice were housed. This housing difference may explain why our mice spent more time on the wheels; simply because the wheel was always easily accessible. Regardless, the selected mice ran significantly more than the controls over the course of the eight week experiment, as determined by the automated system and as visually confirmed by the instantaneous scan sampling during the dark portion of the daily cycle.

The body mass of all mice was less during week one when compared to all of the later weeks (which were not different from one another). This may have occurred by the combination of exposing the mice to a novel environment and to the stress of being singly housed in a new cage. Acute stress is known to reduce body mass, but recovery occurs once the animal adapts to the stressor (Harris *et al.,* 1998; Harris *et al.,* 2002), and social isolation increases the susceptibility of weight loss (Boakes and Dwyer, 1997). Control mice with and without wheels did not differ in body mass, but the selected mice with wheels were heavier than those without wheels. The selected mice, which we have established run further, weigh less than the control mice due to genetics, but the increased activity (increased locomotor running activity, but general increased "active" behavior also) may also contribute to the lower weight in selected mice. Within the selected line, mice with wheel access likely weigh more due to increased energy needed to sustain the elevated exercise, and increased muscle mass would be expected in the selected mice with running-wheels. Selected mice with wheel access were observed consuming more during the dark phase of the daily light cycle as compared to selected mice without running-wheels. Overall, the selected mice weigh less than control mice due to genetics, but given a wheel, selected mice tend to bulk up due to the increased exercise.

Behaviorally, the selected line was much more active than the control line, and this difference was even more pronounced when running wheels were present. The increased activity in the selected animals, especially with wheels, is further supported by the very low frequency of idle and sleep behavior during the dark or "active" portion of their light cycle. Expectedly, the selected mice with wheels consumed more as well. Grooming was also the lowest in the selected line, especially when there was access to a running-wheel. In addition to the increased wheel-running as determined by the automated system, the selected line visually ran more during the dark period of the cycle.

The behavioral component of the current research was similar to the one used by Koteja, *et al*. (1999), except that behavior was studied between the lines with or without running wheels. Koteja *et al*. locked a portion of the running-wheels and evaluated behavior only between mice with locked wheels or those free to rotate. This difference in paradigm may result in substantially different behaviors. In addition, three time periods were evaluated during a 24 hour period, compared to only "night" and "day" in this previous study. Mice behavior was scored one hour before the lights turn off, and also two times (middle and the last hour) during the dark or "active" portion of the cycle. Looking at the two portions of the dark phase allowed the evaluation of temporal differences (i.e., do mice cease certain behaviors at the same time?) in behavior during the active part of the cycle.

Not unexpectedly, and consistent with previous study, selected mice were behaviorally more active than the control mice at all time periods of observation (Rhodes *et al*., 2001). The activity during the dark portion of the light cycle was very interesting, especially during the last hour before the lights turned on. At 9 PM significant line,

wheel, and line*wheel interactions were found. Activity behaviors slowed down during this time period in a similar pattern within each line, with control mice and those mice without wheels ceasing activity first. Therefore, wheel access helps maintain heightened activity levels within each line, and selected individuals maintain elevated activity levels for the longest amount of time. This temporal variation in activity behavior supports an altered circadian period in the selected mice, as found by Koteja *et al*., 2003. Corticosterone fluctuations during the dark cycle would be interesting to monitor in these mice, because alterations in B may influence the activity of the mice, particularly because the selected mice sleep less during the dark phase (Meerlo *et al.*, 2002). Also, Bronikowski *et al*., 2001, found that the novelty of an open field eliminates differences in behavioral activity between lines. Other paradigms measuring anxiety levels, such as the elevated-plus maze or the light-dark box would also be valuable to evaluate with these mice. As a consequence of increased active behavior during the dark phase of the cycle, selected mice had less time available for sleeping. A converse pattern was found for sleep behavior relative to the active behavior during the dark portion of the cycle described above. A very similar pattern was also found for idle behavior. Using similar reasoning, during the dark phase selected mice had less time available for grooming behavior. Often, grooming behavior is an indication of stress or anxiety levels (Kalueff and Touhimaa, 2005), and interestingly, selected mice with wheels exhibited the least amount of grooming during the dark phase, especially during the last hour. Lowered grooming behavior may also be due to the increased amount of time spent engaged in active behaviors. Alternatively, reduced grooming may also be indicative of reduced anxiety levels in the selected mice with wheel access (Fulk *et al.*, 2004). During the dark cycle,

non-wheel selected mice groomed comparable amounts with both control groups, especially at 9 PM. However, the low grooming in the selected mice with wheels may indicate low anxiety levels in this group, suggesting running-wheels may reduce anxiety behavior in the selected line (Dishman, 1997; Fulk *et al.*, 2004; Binder *et al.*, 2004). Overall, selection for increased voluntary wheel-running increases general active behaviors which may be temporally shifted due to altered circadian rhythms, and selection may also alter anxiety levels.

Brain Monoamines:

Very interestingly, we found substantial differences between the lines in basal monoamine activity in several regions of the brain (refer to Table 1). Basal monoamine activity indicates mice were not exposed to running-wheels or a stressor. The selected mice showed an overall reduction dopaminergic and serotonergic activity (DA and/or DOPAC levels were reduced; or 5-HT and/or 5-HIAA levels were reduced, respectively). There were a number of marked neurotransmitter effects in brain regions associated with locomotor activity, emotive status, learning, and motivation/reward. Striatal dopamine levels increase with activity, and increases in DA levels in the anterior caudate nucleus (a portion of the striatum) were also found in selected mice. This is a region rich in DA terminals originating from cell bodies in the SN (Gulley *et al.*, 1999). In both the SN and the VTA (both cell body regions for DA neurons) reduced DA and DOPAC concentrations were evident in selected mice. This indicates lower dopaminergic activity at DA cell body regions in the brains of selected mice and also an increase in terminal production/storage in the anterior caudate which may be compensating for the reduced dopaminergic activity in the SN. This alteration in the nigrostriatal system of selected

mice may allow them to run more, when given the motivational cue of a running-wheel. Interestingly, the levels of both DA and DOPAC were also reduced in the dRN, which is a cell body region for the production of serotonin.

Also in the dRN, 5-HT levels were reduced in the selected line of mice. Reciprocal activity of DA and 5-HT systems may be working through the dRN in the selected mice, and motivational serotonergic circuitry may be linked to the nigrostriatal DA system via the dRN, which would explain the increased running activity in these mice (Martin-Ruiz *et al.*, 2001). Further supporting this reciprocal activity between the DA and 5-HT cell body regions, metabolite levels of serotonin, or 5-HIAA, were also reduced in the selected mice in these same (SN, VTA, dRN) and also in additional brain regions (refer to Table 1).

Regions of the brain linked to emotive state, learning, and motivation/reward also showed reduced serotonergic activity in the selected mice. Both 5-HT and 5-HIAA levels were reduced in the medial amygdala of selected mice, which may explain the emotional mental state of these behaviorally and physically active mice. This reduced serotonergic function within the amygdala may also explain the increased aggression in the selected line (Gammie *et al.*, 2003). Also, two regions of the hippocampus (both CA_1 and CA_3) showed reduced levels of metabolite (5-HIAA), which indicates lower serotonergic activity in a region associated with learning. Reduced serotonergic activity in these nuclei may be responsible for the learning deficit that has been found in the selected mice, even though increased BDNF and neurogenesis was found in the hippocampus (Rhodes *et al.*, 2003b). The nucleus accumbens is an important reward and motivational linked region of the brain. This region also showed lower serotonergic

function, via lower levels of 5-HIAA. The reduced serotonergic activity in both the lateral and medial n. accumbens may be linked to the heightened motivational drive in the selected mice toward extensive exercise. This alteration in the nucleus accumbens of the selected mice can provide the drive for the goal-oriented wheel-running behavior. This heightened motivation to run caused by reduced serotonergic activity in the accumbens may be executed by linking the 5-HT system through the dRN to the nigrostriatal system DA system. The striatum has elevated DA levels which can support the increased exercise desire of the selected mice.

It is important to note that these mice have not had wheel exposure; therefore, the alterations found in the brain systems should be genetically induced, not environmentally. The serotonergic alterations in the above mentioned brain regions may also be important for the study of anxiety. Therefore, these regions should be examined with future study of anxiety paradigms with these mice. Overall, we found that both dopaminergic and serotonergic function are decreased in mice selected for increased voluntary wheel-running, and these brain alterations may explain the behavioral, emotional, and activity differences in these mice.

Conclusions:

Selection for an increased propensity to exercise results in increased active behavior. And, selected mice with wheels groomed very little compared to all other groups during the most active portion of the day, indicating possibly lower anxiety levels. Monaminergic activity was lowered in mice selected for increased voluntary wheel-running, as indicated by both lower dopaminergic and lower serotonergic functioning in the brain. Dopamine was higher in the striatum of selected mice, which explain how the

selected mice run more. Also, the lower serotonergic activity in the accumbens explains why the selected mice can be more motivated to exercise on a running-wheel. Interesting future studies would be to use microdialysis experiments to manipulate the putative altered systems in the brain and to also use receptor-staining via immunocytochemistry to quantify 5-HT and DA receptor levels. Also, a critical evaluation of various anxiety paradigms or an ADHD test on these mice should be warranted. Future publications will include monoaminergic responses to an acute stress test with these mice.

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Table and Figure Legends:

Table 1: Basal monamine results when comparing control vs. selected mice as analyzed via student's t-test. Twelve brain regions were analyzed for concentrations of NE, Epi, DOPAC, DA, 5-HT, 5-HIAA (metabolite of 5-HT), and the ratios of the neurotransmitter to its metabolite (DOPAC/DA and 5-HIAA/5-HT). These ratios are often useful to interpret metabolic turnover and help to understand dopaminergic and serotonergic activity. Brain regions are listed on the left, followed by monoamine results (T-values, P-values, and DF when comparing control vs. selected mice. An alpha of $P<0.05$ is considered significant.

Figure 1: Wheel-running activity (distance run in meters) as analyzed via ANOVA. Over the eight weeks, mice selected for voluntary wheel-running with access to an activity wheel [12,811.18 $\pm$ 258.66 (SEM) m/day] ran 3.02 times further than the control mice with wheels [4,246.98 $\pm$ 167.13 m/day; $F_{1,1294}=773.39$, $P<0.001$ (* in the inserted graph)]. The large graph illustrates the mean (w/SEM) daily distance run in meters for the control and selected mice. The arrow near the x-axis indicates the commencement of the behavioral observations.

Figure 2: Wheel-running activity (running speed in m/min) as analyzed via ANOVA. Selected mice (23.97 $\pm$ 0.364 m/min) ran 1.60 times faster than control mice [14.95 $\pm$ 0.339 m/min; $F_{1,1294}=328.50$, $P<0.001$ (* in the inserted graph)]. The large graph illustrates the mean (w/SEM) running speed in meters per minute for the control and

selected mice. The arrow near the x-axis indicates the commencement of the behavioral observations.

Figure 3: Wheel-running activity (time spent running in minutes) as analyzed via ANOVA. Selected mice spent 2.23 times more time (507.27 $\pm$ 5.9 min/day) actively engaged in running on the wheels than control mice [227.94 $\pm$ 5.84 min/day; $F_{1,1294}$=1132.05, P<0.001 (* in the inserted graph)]. The large graph illustrates the mean (w/SEM) time spent running in minutes for the control and selected lines of mice. The arrow near the x-axis indicates the commencement of the behavioral observations.

Figure 4: Body mass (grams) of control and selected mice with and without running-wheel access as analyzed via ANOVA. All mice weighed significantly less during week one when compared to later weeks ($F_{8,450}$=5.263, P<0.001). Throughout the eight week experiment, control (38.71 $\pm$ 0.174) mice weighed significantly more than selected mice (34.34 $\pm$ 0.202; $F_{1,450}$=275.83, P<0.001). Overall, mice with access to a wheel were significantly heavier than those without ($F_{1,450}$=6.032, P=0.014). A significant line by wheel interaction was found such that selected mice with access to running-wheels (35.03 $\pm$ 0.241) were heavier than those without (33.75 $\pm$ 0.304; $F_{1,450}$=5.919, P=0.015).

Figure 5: Histogram of the ethological observations during the last four weeks of the experiment. Behaviors were scored in small categories (example: eat, drink, stand/sniff, wheel-run, etc.) and then later lumped into the following mutually exclusive ethological categories: “groom” (licking at fur and rubbing paws), “sleep” (curled up with eyes

closed), “idle” (sitting motionless with eyes open), “consumption” (in the motions of eating or drinking), and “active” (moving in cage, running, standing/sniffing, or hanging from wire cage top). At all observation periods, selected mice were more active than the control mice ($P<0.001$). Also, at 9 PM, W mice overall and within each line were more active than NW mice ($P<0.002$). At 3 PM and 9 PM, S mice and W mice were much less idle than C and NW mice ($P<0.001$). At all 3 times, S mice slept significantly less than C mice ($P<0.001$). In general, W mice sleep less than NW mice.

Figure 6: *Active behavior* as analyzed via ANOVA. An * indicates $P<0.05$, and a # indicates $P<0.08$. Line = control vs. selected mice; Activity = wheel vs. non-wheel: Line*Activity is the interaction. The last hour of light for the mice was from 9-10 AM. The mid-portion of the dark cycle (when mice are typically most active) was at 3 PM, and 9-10 PM was the last hour of darkness for the animals.

Figure 7: *Consumption behavior* as analyzed via ANOVA. An * indicates $P<0.05$, and a # indicates $P<0.08$. Line = control vs. selected mice; Activity = wheel vs. non-wheel: Line*Activity is the interaction. The last hour of light for the mice was from 9-10 AM. The mid-portion of the dark cycle (when mice are typically most active) was at 3 PM, and 9-10 PM was the last hour of darkness for the animals.

Figure 8: *Idle behavior* as analyzed via ANOVA. An * indicates $P<0.05$, and a # indicates $P<0.08$. Line = control vs. selected mice; Activity = wheel vs. non-wheel: Line*Activity is the interaction. The last hour of light for the mice was from 9-10 AM.

The mid-portion of the dark cycle (when mice are typically most active) was at 3 PM, and 9-10 PM was the last hour of darkness for the animals.

Figure 9: *Sleep behavior* as analyzed via ANOVA. An * indicates P<0.05, and a # indicates P<0.08. Line = control vs. selected mice; Activity = wheel vs. non-wheel: Line*Activity is the interaction. The last hour of light for the mice was from 9-10 AM. The mid-portion of the dark cycle (when mice are typically most active) was at 3 PM, and 9-10 PM was the last hour of darkness for the animals.

Figure 10: *Grooming behavior* as analyzed via ANOVA. An * indicates P<0.05, and a # indicates P<0.08. Line = control vs. selected mice; Activity = wheel vs. non-wheel: Line*Activity is the interaction. The last hour of light for the mice was from 9-10 AM. The mid-portion of the dark cycle (when mice are typically most active) was at 3 PM, and 9-10 PM was the last hour of darkness for the animals.

Figure 11: *Wheel-running behavior* between control and selected mice as analyzed via ANOVA. An * indicates P<0.05. The last hour of light for the mice was from 9-10 AM. The mid-portion of the dark cycle (when mice are typically most active) was at 3 PM, and 9-10 PM was the last hour of darkness for the animals.

Figure 12: Basal plasma corticosterone (B) levels comparing control vs. selected mice as analyzed by student's t-test. Corticosterone levels did not differ between the selected and control mice (t=0.627, P=0.541).

Figure 13: Basal NE concentrations comparing control vs. selected mice for twelve brain regions as analyzed by student's t-test. An * indicates $P<0.05$, and a # indicates $P<0.08$.

Figure 14: Basal Epi concentrations comparing control vs. selected mice for twelve brain regions as analyzed by student's t-test. An * indicates $P<0.05$, and a # indicates $P<0.08$.

Figure 15: Basal DOPAC concentrations comparing control vs. selected mice for twelve brain regions as analyzed by student's t-test. An * indicates $P<0.05$, and a # indicates $P<0.08$.

Figure 16: Basal DA concentrations comparing control vs. selected mice for twelve brain regions as analyzed by student's t-test. An * indicates $P<0.05$, and a # indicates $P<0.08$.

Figure 17: Basal ratios of DOPAC/DA comparing control vs. selected mice for twelve brain regions as analyzed by student's t-test. An * indicates $P<0.05$, and a # indicates $P<0.08$.

Figure 18: Basal 5-HIAA concentrations comparing control vs. selected mice for twelve brain regions as analyzed by student's t-test. An * indicates $P<0.05$, and a # indicates $P<0.08$.

Figure 19: Basal 5-HT concentrations comparing control vs. selected mice for twelve brain regions as analyzed by student's t-test. An * indicates $P<0.05$, and a # indicates $P<0.08$.

Figure 20: Basal ratios of 5-HIAA/5-HT comparing control vs. selected mice for twelve brain regions as analyzed by student's t-test. An * indicates $P<0.05$, and a # indicates $P<0.08$.

Tables and Figures:

Table 1

C vs. S		NE	EPI	DOPAC	DA	DOPAC/DA	5-HIAA	5-HT	5-HIAA/5-HT
dRN	T val.	3.263	0.382	2.715	3.959	-1.804	3.203	2.153	0.013
	P val.	0.006	0.708	0.017	0.002	0.095	0.007	0.051	0.990
	DF	13	13	13	13	13	13	13	13
aCPu	T val.	-0.040	-0.459	-0.146	-2.655	0.764	1.327	3.377	-1.165
	P val.	0.968	0.653	0.886	0.019	0.458	0.207	0.004	0.265
	DF	13	13	13	13	13	13	13	13
Hip(CA_1)	T val.	1.719	-0.147	-0.072	0.069	-0.287	2.165	1.308	0.657
	P val.	0.111	0.885	0.943	0.945	0.778	0.049	0.213	0.522
	DF	12	11	13	12	12	13	13	13
Hip(CA_3)	T val.	1.947	-1.460	0.746	-0.521	-0.153	2.226	-0.519	1.002
	P val.	0.073	0.167	0.468	0.611	0.880	0.044	0.612	0.334
	DF	13	13	13	12	13	13	12	13
LC	T val.	0.278	-3.396	0.959	1.114	0.655	0.704	-0.338	3.897
	P val.	0.784	0.005	0.355	0.285	0.523	0.493	0.740	0.001
	DF	13	12	13	13	13	13	12	13
AME	T val.	1.557	2.110	-1.151	-0.563	0.891	2.622	2.300	0.758
	P val.	0.143	0.050	0.270	0.583	0.393	0.021	0.038	0.461
	DF	13	13	13	12	10	13	13	13
CeA	T val.	1.898	-0.973	1.275	-0.358	1.033	0.708	-0.422	1.183
	P val.	0.081	0.348	0.224	0.726	0.320	0.491	0.679	0.257
	DF	13	13	13	13	13	13	13	13
lNacc	T val.	0.887	-1.256	0.932	-0.100	0.236	2.199	1.479	0.422
	P val.	0.391	0.231	0.368	0.921	0.816	0.050	0.162	0.679
	DF	13	13	13	13	13	13	13	13
mNacc	T val.	2.145	0.597	0.408	0.301	0.167	4.647	1.413	0.357
	P val.	0.051	0.560	0.689	0.768	0.869	0.0004	0.180	0.726
	DF	13	13	13	13	13	13	13	13
PVN	T val.	-2.683	0.563	0.666	0.576	0.172	1.640	0.749	1.400
	P val.	0.019	0.582	0.516	0.574	0.865	0.124	0.467	0.184
	DF	13	13	13	13	13	13	13	13
SN	T val.	-0.347	-0.840	3.951	2.024	2.069	3.003	0.943	1.620
	P val.	0.734	0.416	0.001	0.063	0.058	0.010	0.362	0.129
	DF	13	12	13	13	13	13	13	13
VTA	T val.	1.888	-0.576	3.892	3.003	-1.310	3.282	1.375	1.145
	P val.	0.082	0.573	0.001	0.010	0.212	0.005	0.192	0.272
	DF	13	13	13	13	13	13	13	13

Figure 1

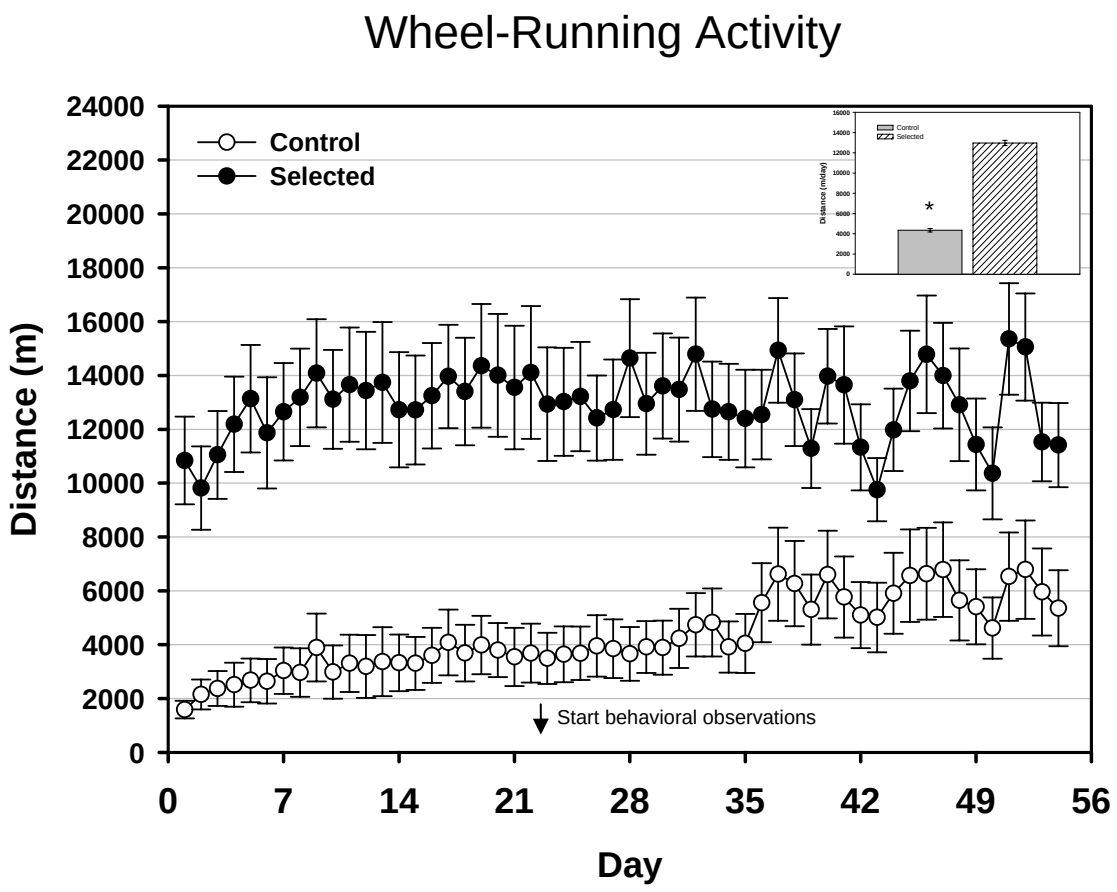

Wheel-Running Activity
Control
Selected
Distance (m)
Day
Start behavioral observations
Distance (m/day)
*

Figure 2

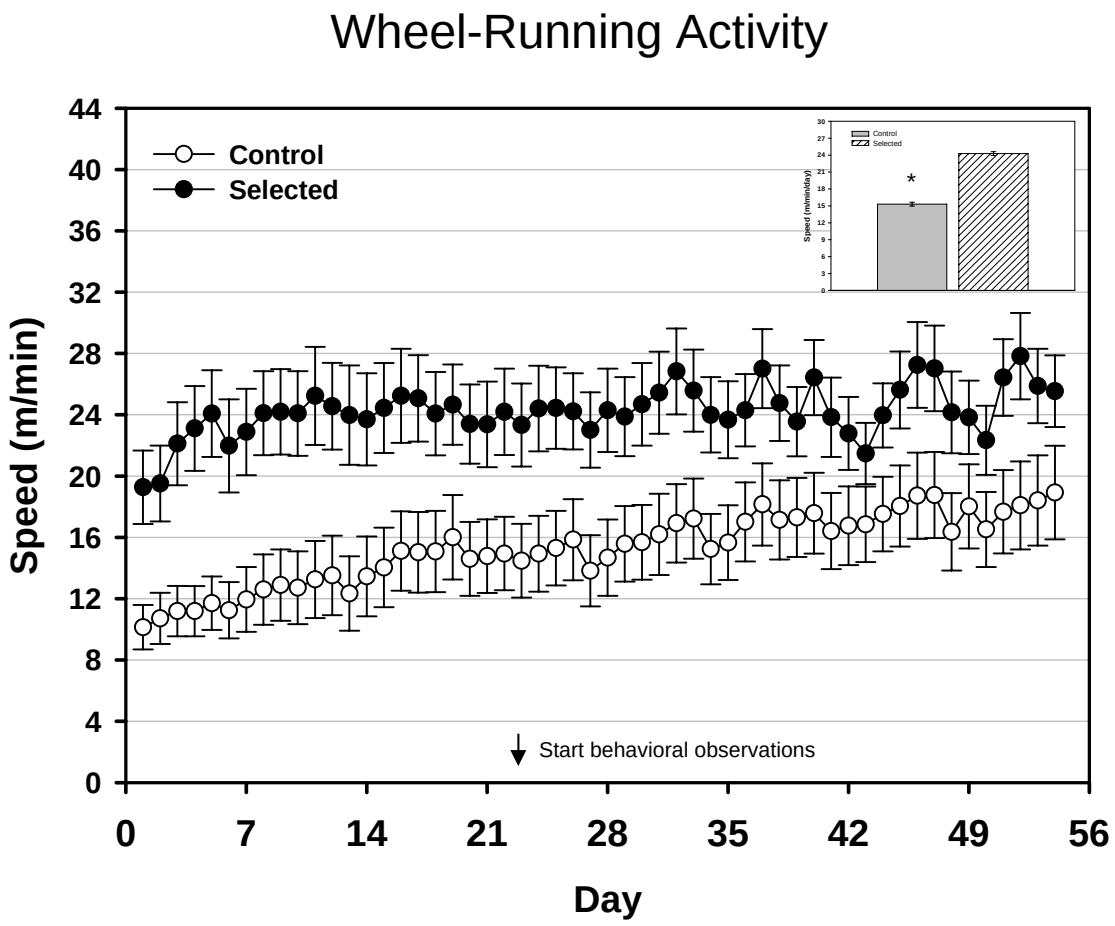

Wheel-Running Activity
Control
Selected
Speed (m/min)
Day
Start behavioral observations
*

Figure 3

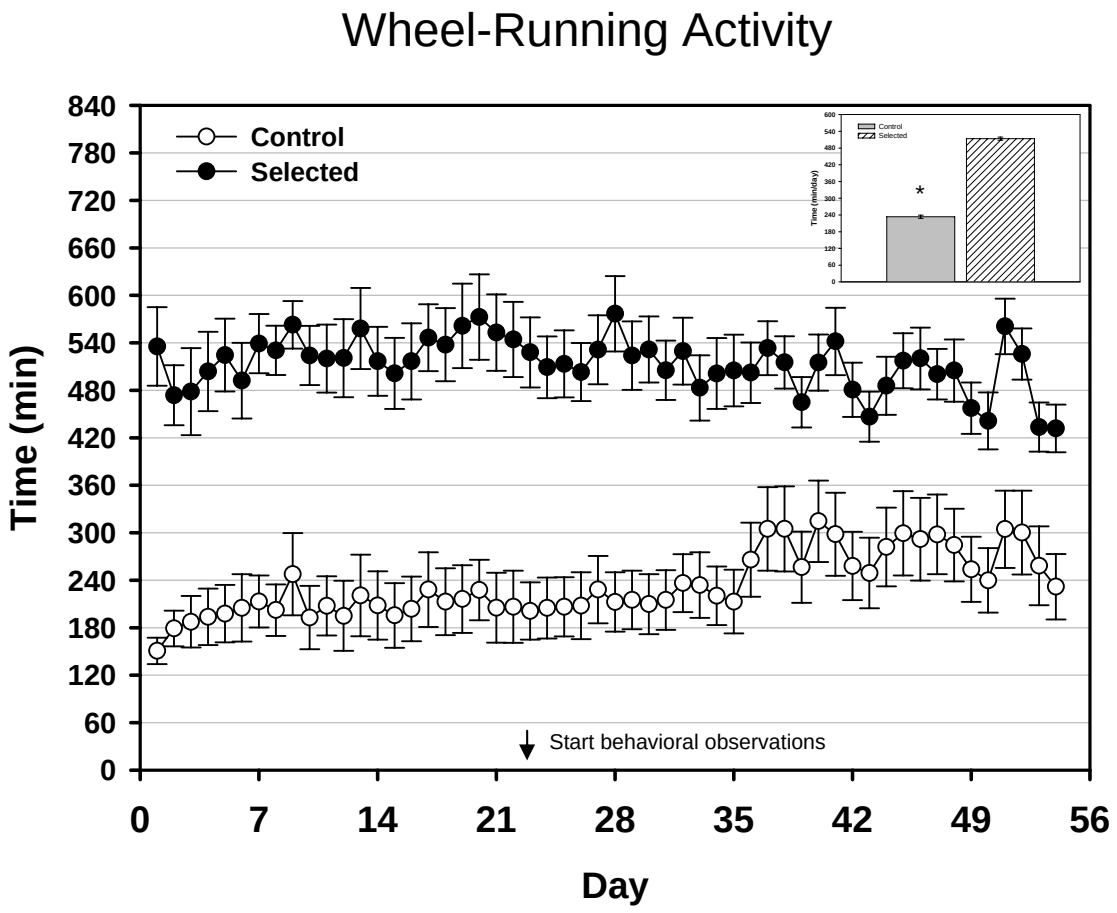

Wheel-Running Activity
Control
Selected
Time (min)
Day
Start behavioral observations
Time (min/day)
*
*

Figure 4

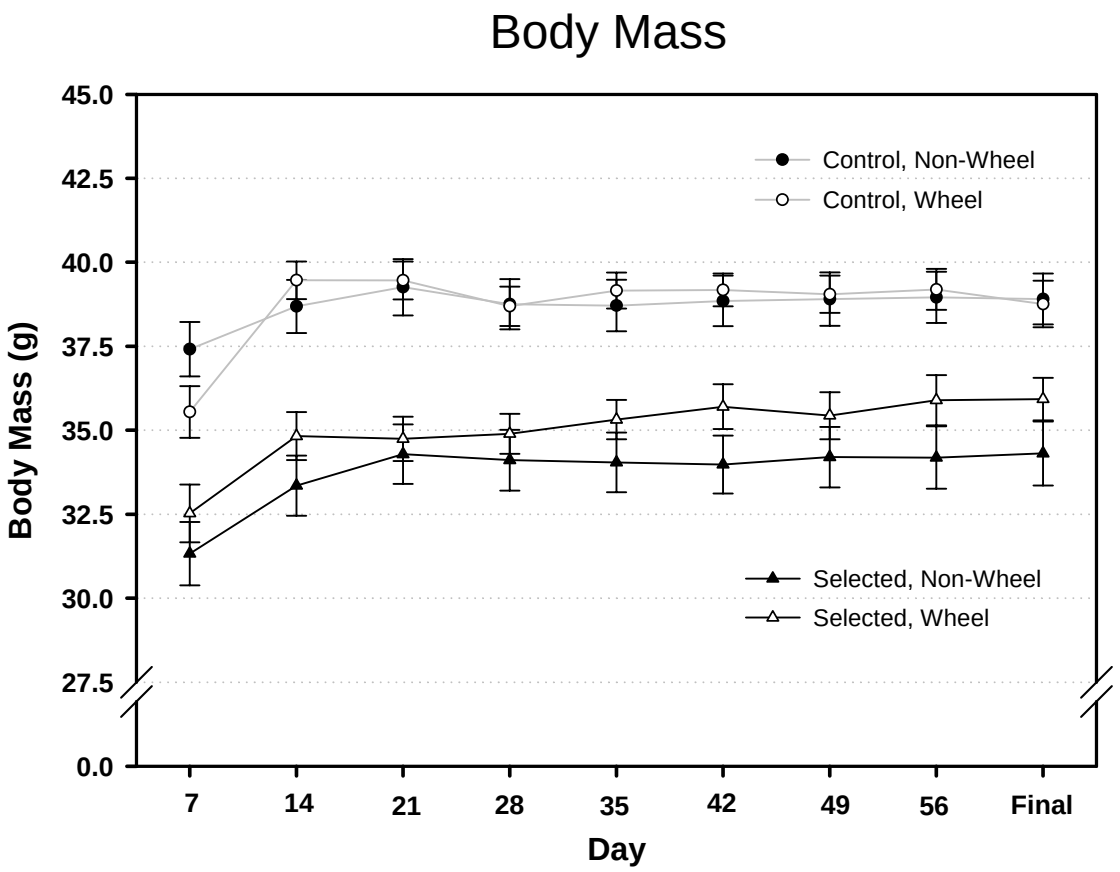

Body Mass
45.0
42.5
40.0
37.5
35.0
32.5
30.0
27.5
0.0
Body Mass (g)
Control, Non-Wheel
Control, Wheel
Selected, Non-Wheel
Selected, Wheel
7
14
21
28
35
42
49
56
Final
Day

Figure 5

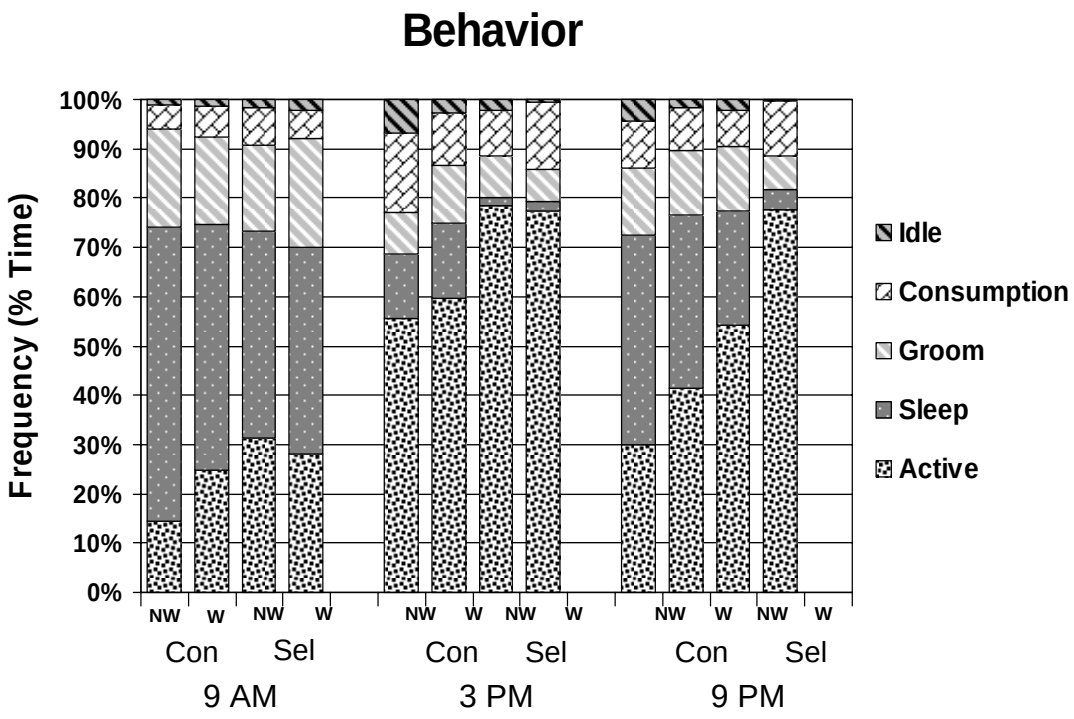

Behavior
Frequency (% Time)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
NW
W
Con
Sel
9 AM
3 PM
9 PM
Idle
Consumption
Groom
Sleep
Active

Figure 6

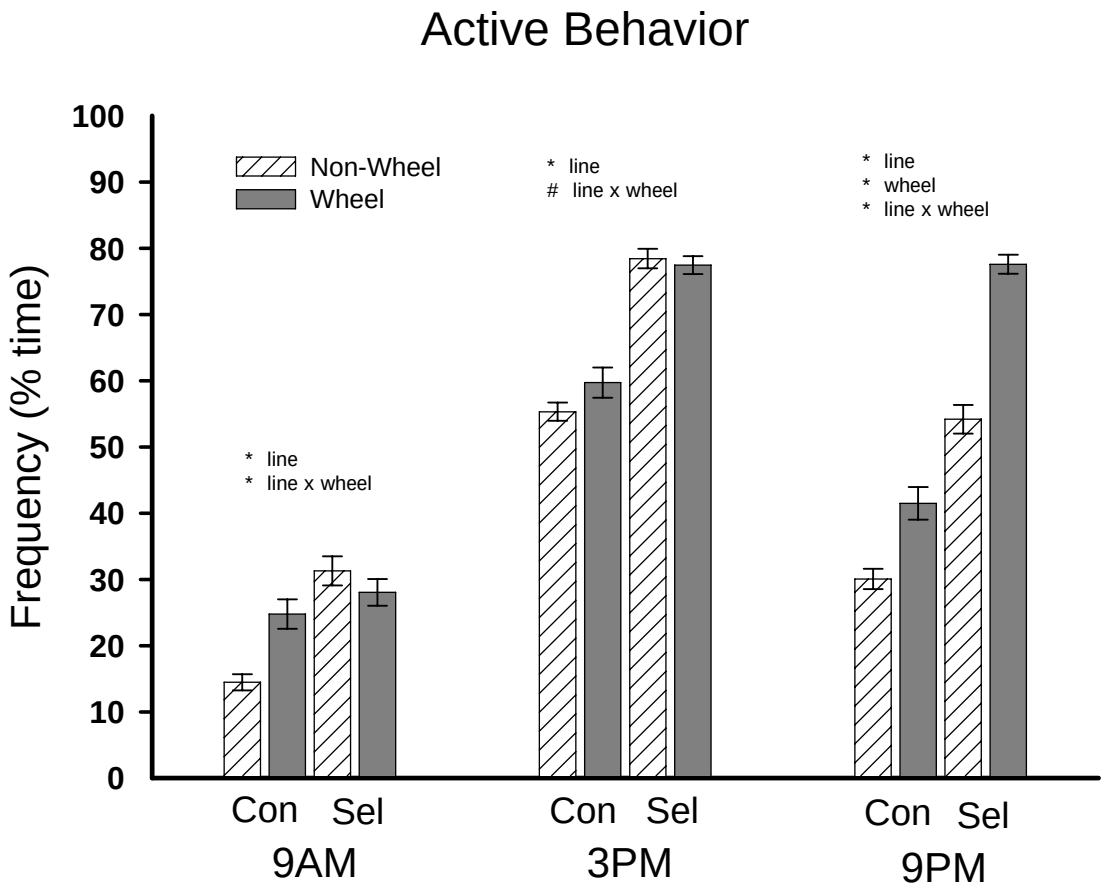

Active Behavior
Frequency (% time)
100
90
80
70
60
50
40
30
20
10
0
Non-Wheel
Wheel
* line
* line x wheel
* line
# line x wheel
* line
* wheel
* line x wheel
Con Sel
9AM
Con Sel
3PM
Con Sel
9PM

Figure 7

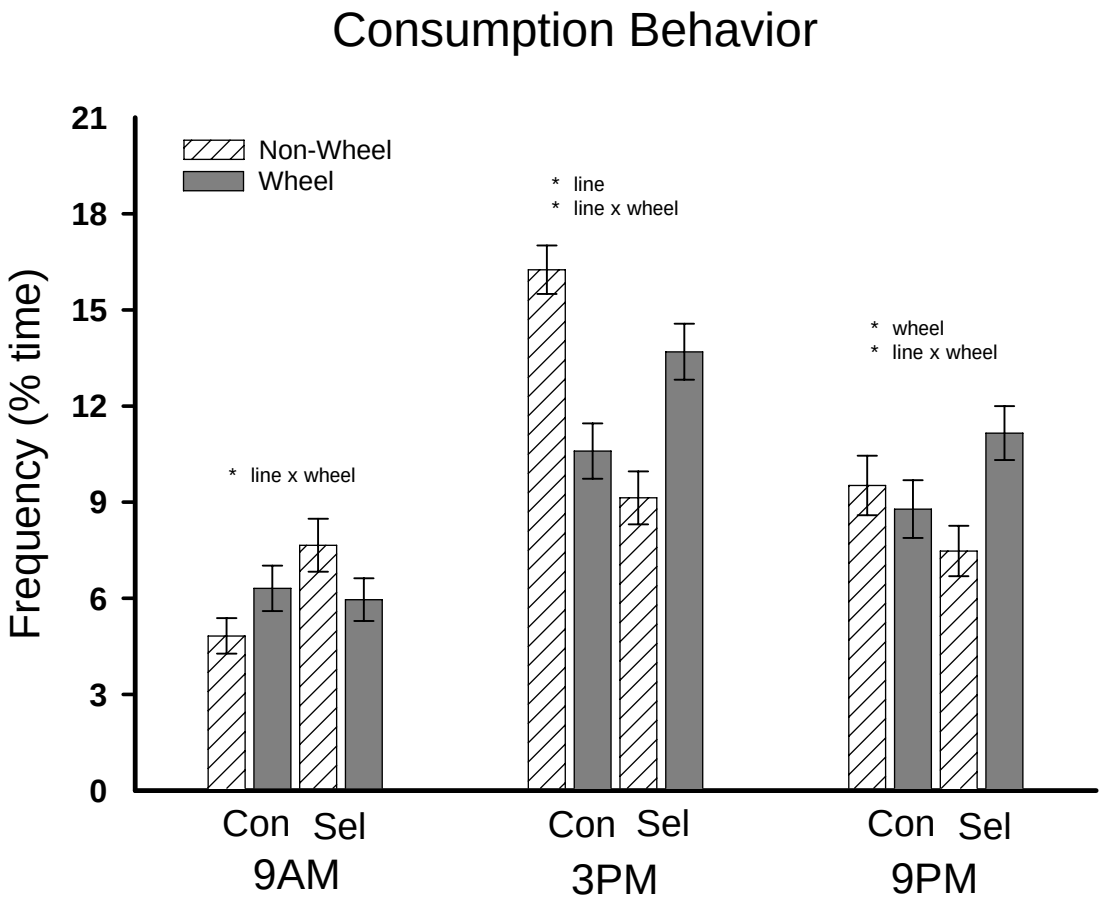

Consumption Behavior
Frequency (% time)
21
18
15
12
9
6
3
0
Non-Wheel
Wheel
* line x wheel
* line
* line x wheel
* wheel
* line x wheel
Con Sel
9AM
Con Sel
3PM
Con Sel
9PM

Figure 8

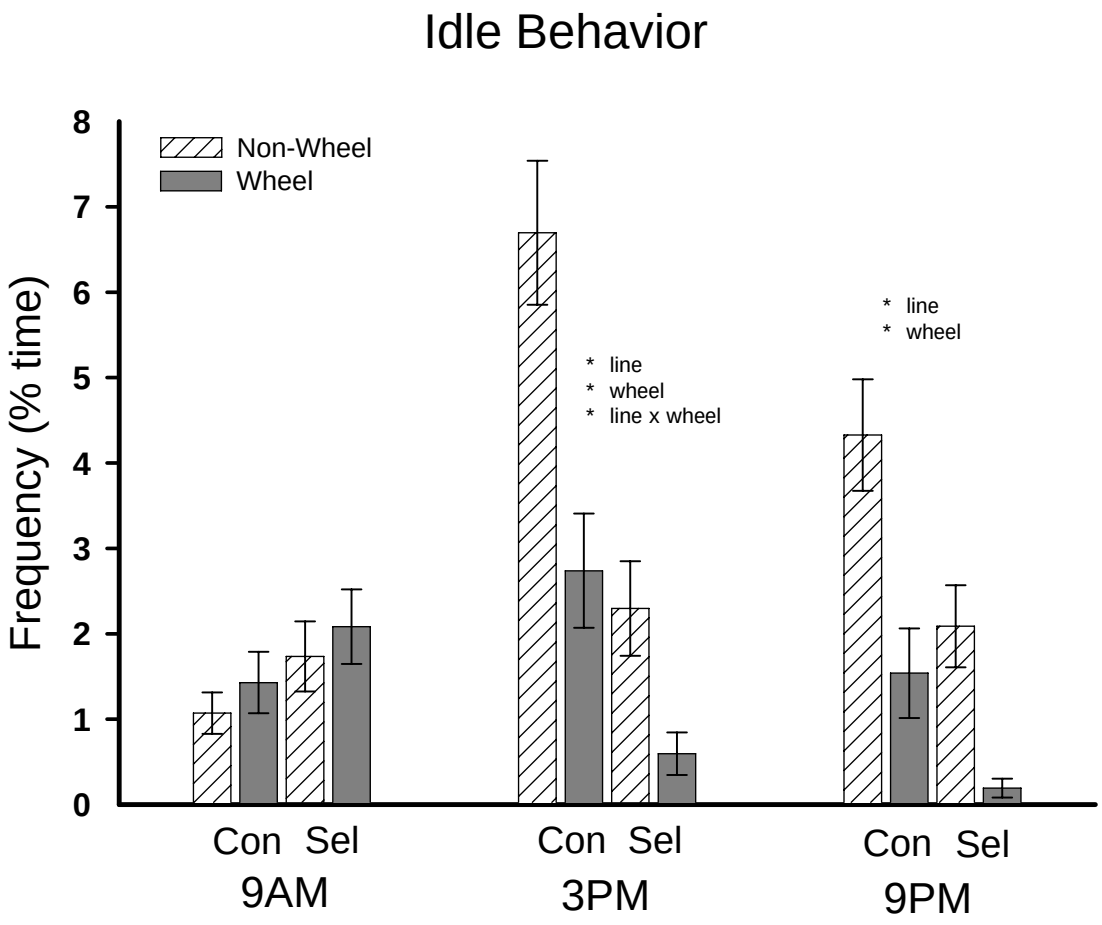

Idle Behavior
Non-Wheel
Wheel
Frequency (% time)
0
1
2
3
4
5
6
7
8
* line
* wheel
* line x wheel
* line
* wheel
Con Sel
9AM
Con Sel
3PM
Con Sel
9PM

Figure 9

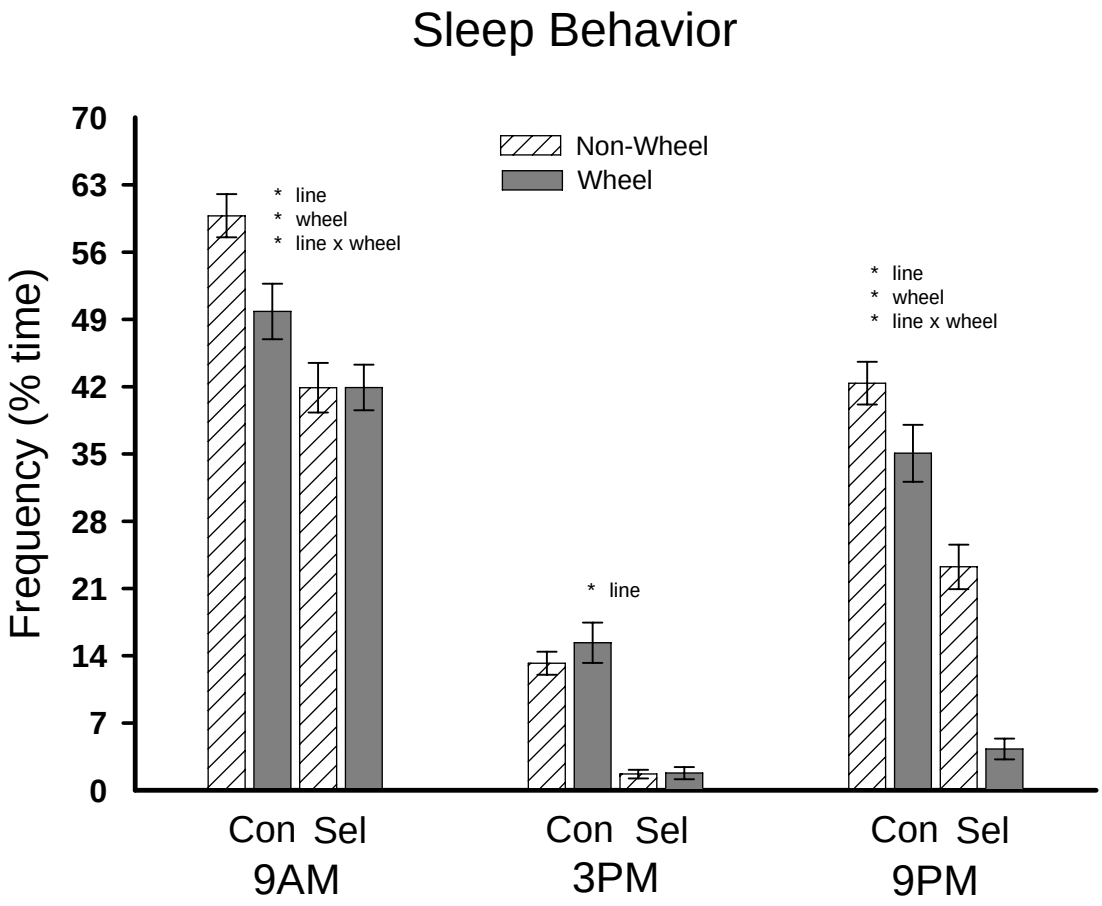

Sleep Behavior
Frequency (% time)
70
63
56
49
42
35
28
21
14
7
0
Non-Wheel
Wheel
* line
* wheel
* line x wheel
* line
* line
* wheel
* line x wheel
Con Sel
9AM
Con Sel
3PM
Con Sel
9PM

Figure 10

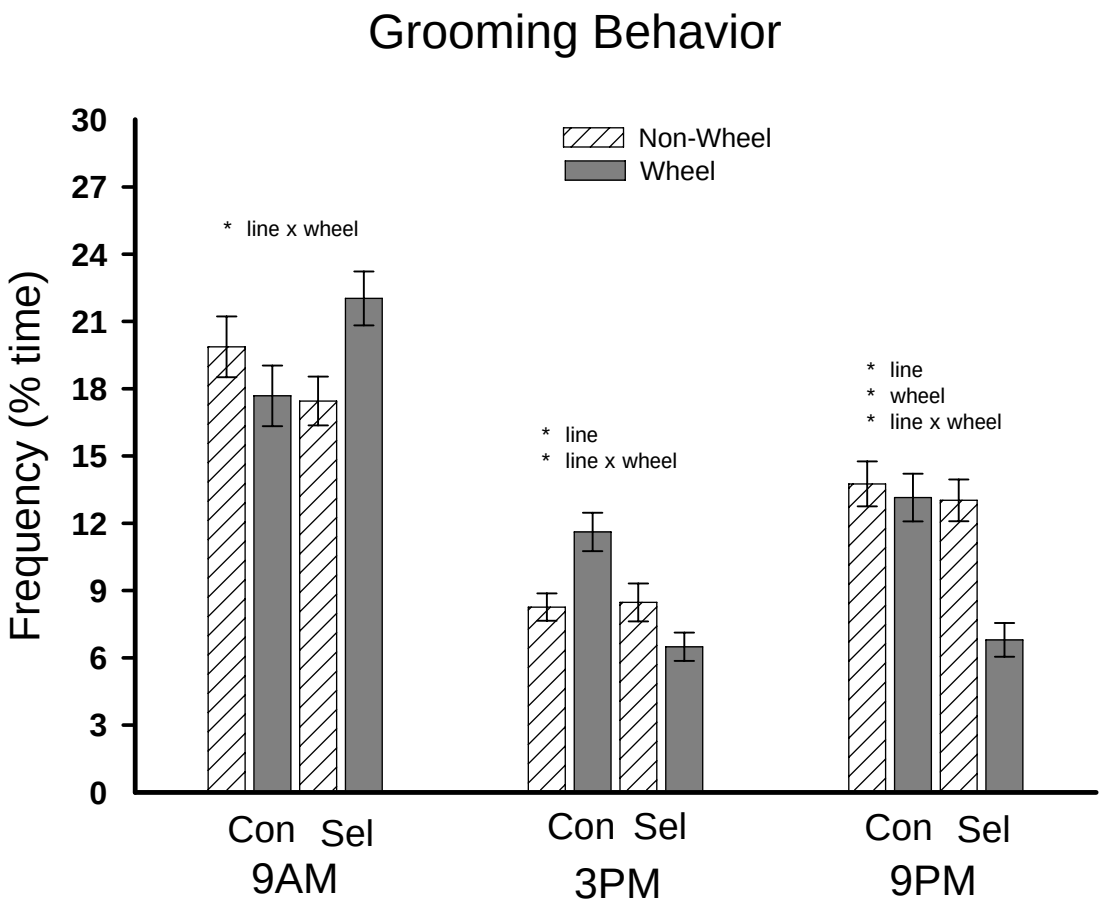

Grooming Behavior
Non-Wheel
Wheel
* line x wheel
* line
* line x wheel
* line
* wheel
* line x wheel
Frequency (% time)
30
27
24
21
18
15
12
9
6
3
0
Con Sel
9AM
Con Sel
3PM
Con Sel
9PM

Figure 11

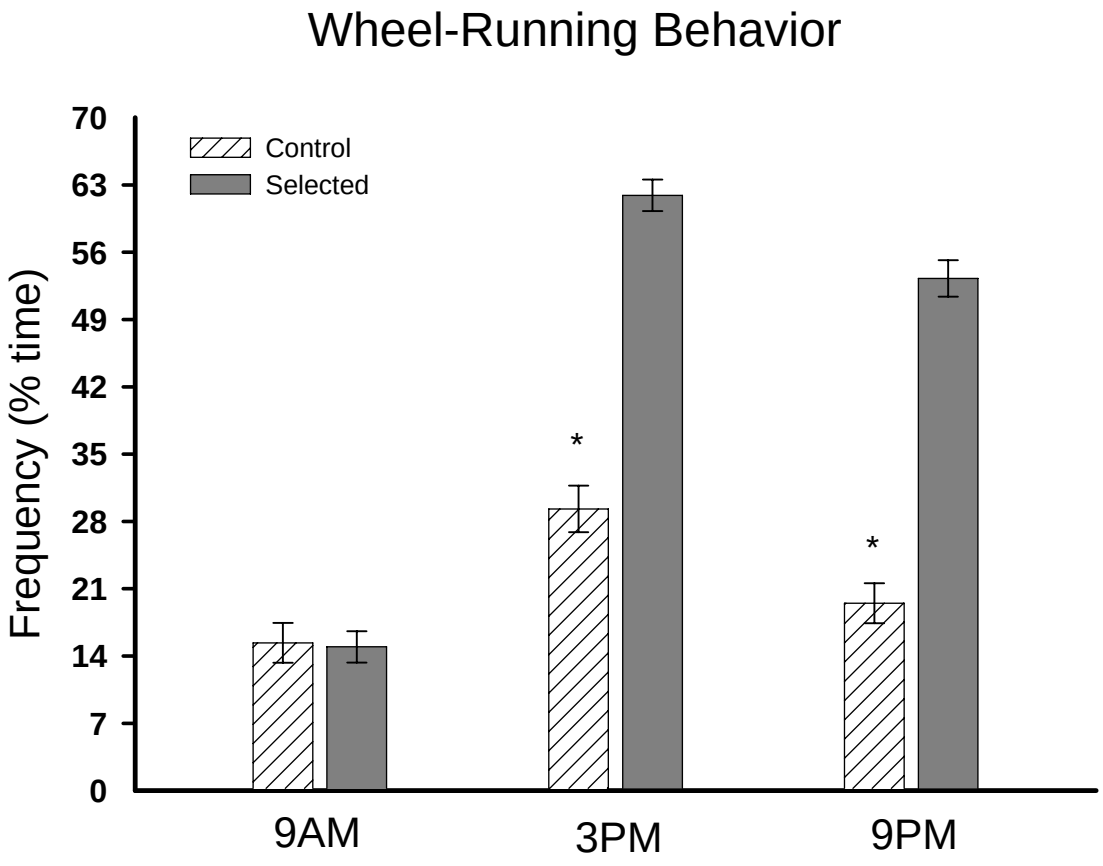

Wheel-Running Behavior
Frequency (% time)
70
63
56
49
42
35
28
21
14
7
0
Control
Selected
*
*
9AM
3PM
9PM

Figure 12

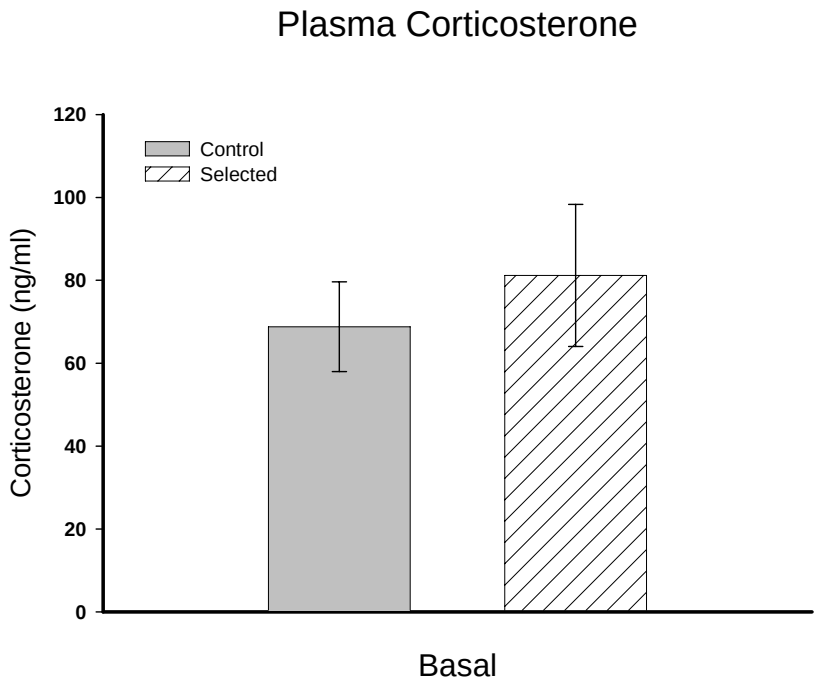

Plasma Corticosterone
Control
Selected
Corticosterone (ng/ml)
120
100
80
60
40
20
0
Basal

Figure 13

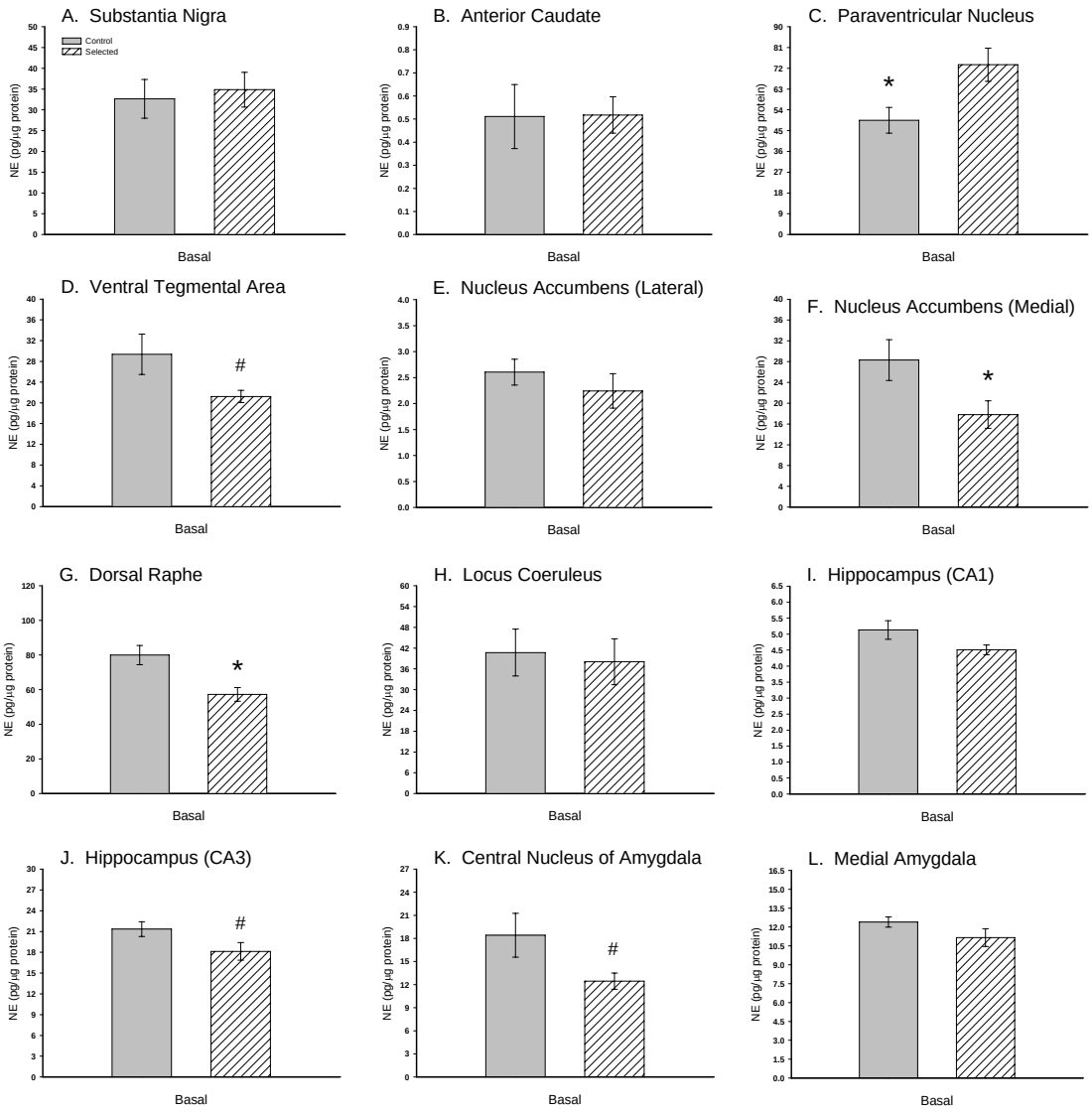

A. Substantia Nigra
Control
Selected
B. Anterior Caudate
C. Paraventricular Nucleus
D. Ventral Tegmental Area
E. Nucleus Accumbens (Lateral)
F. Nucleus Accumbens (Medial)
G. Dorsal Raphe
H. Locus Coeruleus
I. Hippocampus (CA1)
J. Hippocampus (CA3)
K. Central Nucleus of Amygdala
L. Medial Amygdala
NE (pg/µg protein)
Basal
*
#

Figure 14

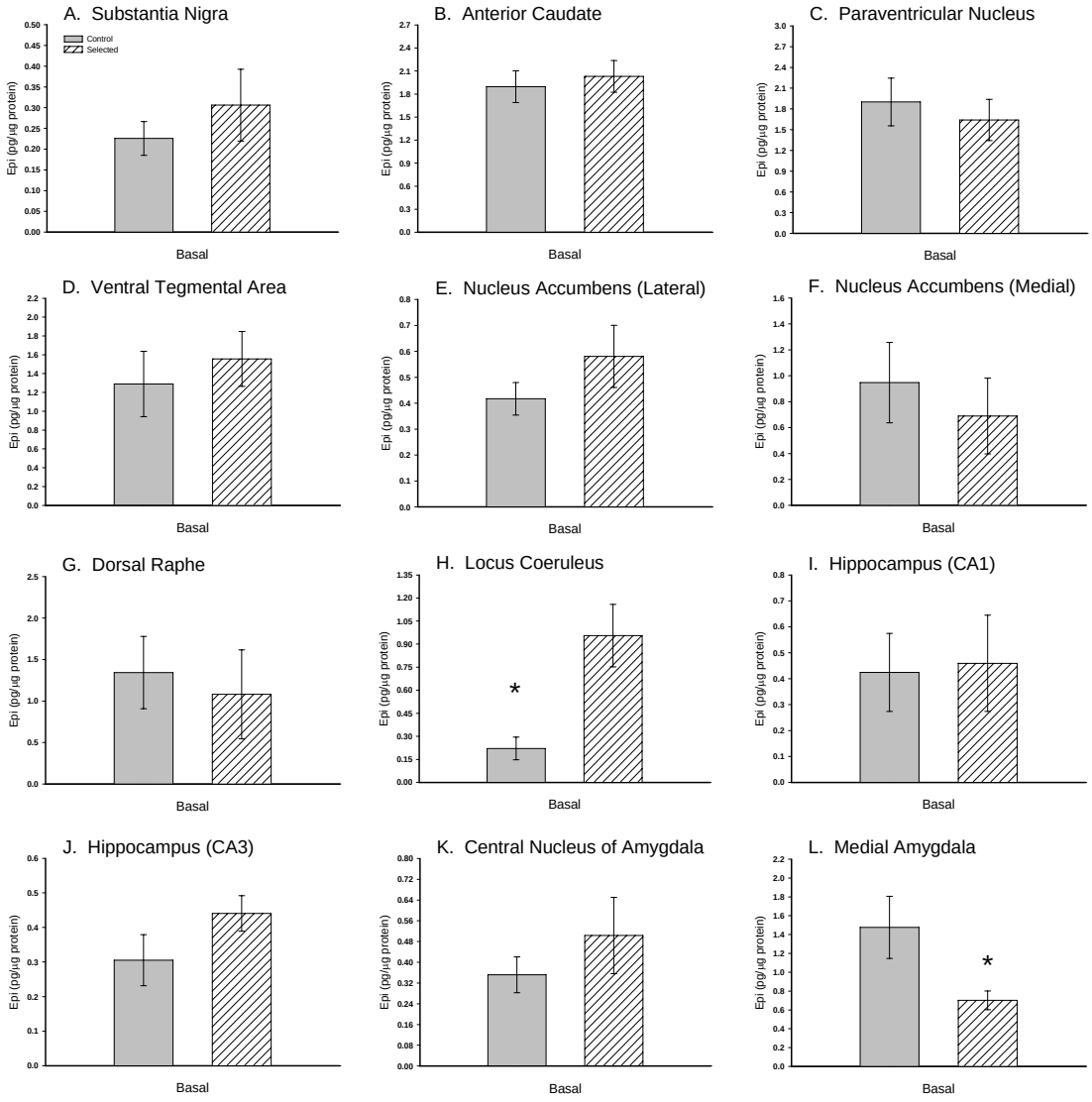

A. Substantia Nigra
Control
Selected
B. Anterior Caudate
C. Paraventricular Nucleus
D. Ventral Tegmental Area
E. Nucleus Accumbens (Lateral)
F. Nucleus Accumbens (Medial)
G. Dorsal Raphe
H. Locus Coeruleus
I. Hippocampus (CA1)
J. Hippocampus (CA3)
K. Central Nucleus of Amygdala
L. Medial Amygdala
Epi (pg/µg protein)
Basal
*

Figure 15

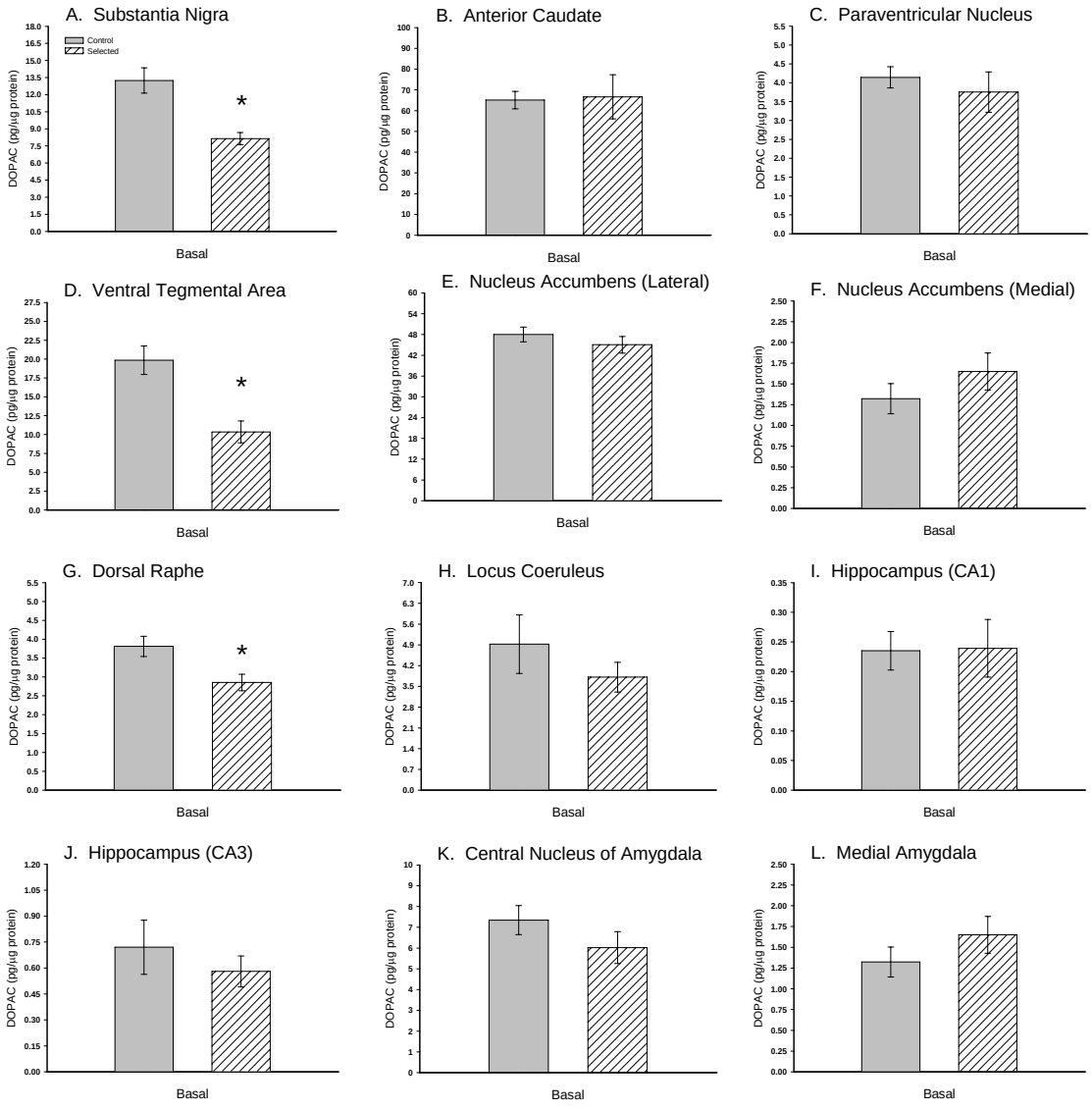

A. Substantia Nigra
Control
Selected
DOPAC (pg/µg protein)
Basal
B. Anterior Caudate
C. Paraventricular Nucleus
D. Ventral Tegmental Area
E. Nucleus Accumbens (Lateral)
F. Nucleus Accumbens (Medial)
G. Dorsal Raphe
H. Locus Coeruleus
I. Hippocampus (CA1)
J. Hippocampus (CA3)
K. Central Nucleus of Amygdala
L. Medial Amygdala

Figure 16

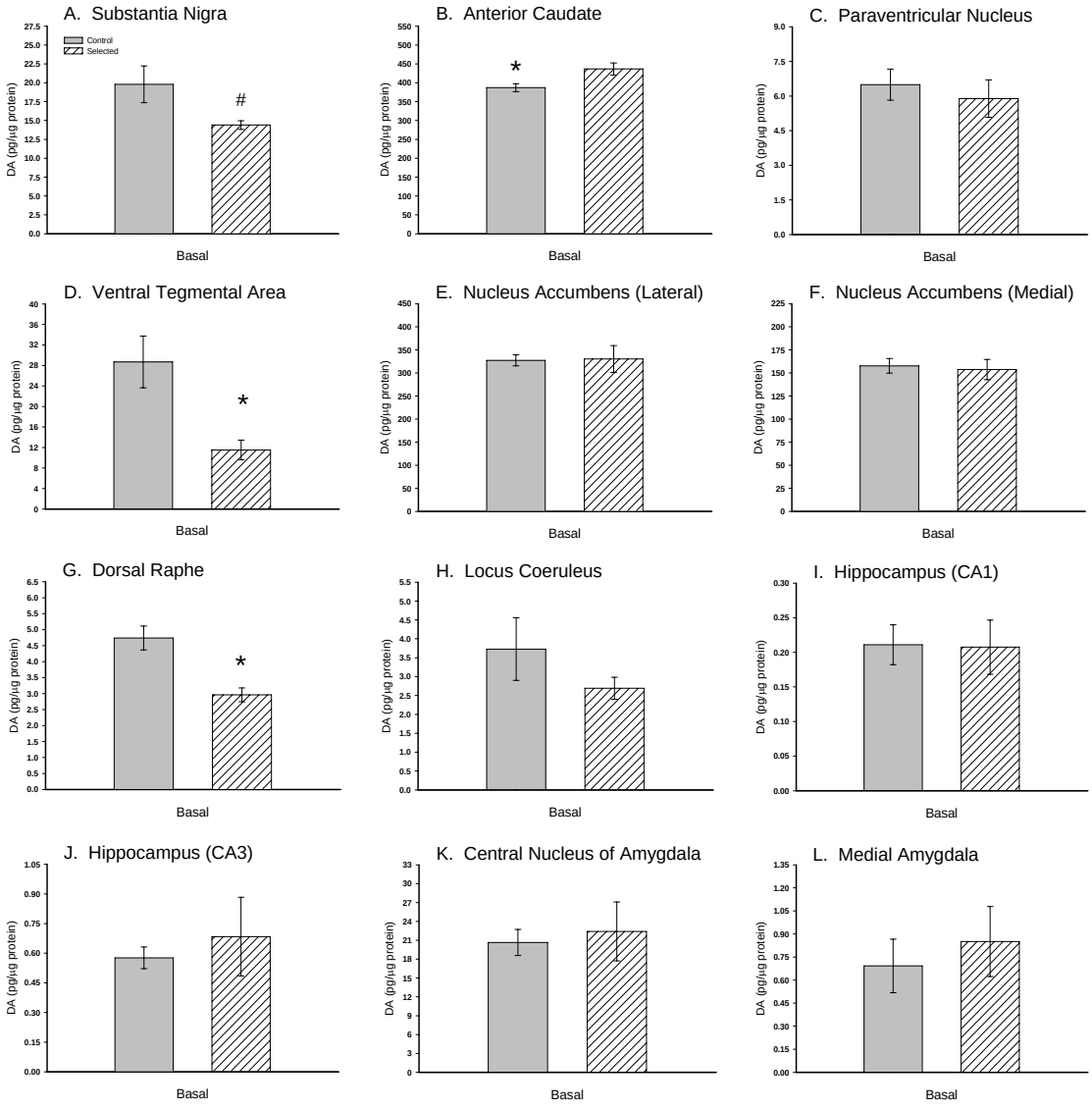

A. Substantia Nigra
Control
Selected
#
B. Anterior Caudate
*
C. Paraventricular Nucleus
D. Ventral Tegmental Area
*
E. Nucleus Accumbens (Lateral)
F. Nucleus Accumbens (Medial)
G. Dorsal Raphe
*
H. Locus Coeruleus
I. Hippocampus (CA1)
J. Hippocampus (CA3)
K. Central Nucleus of Amygdala
L. Medial Amygdala
DA (pg/µg protein)
Basal

Figure 17

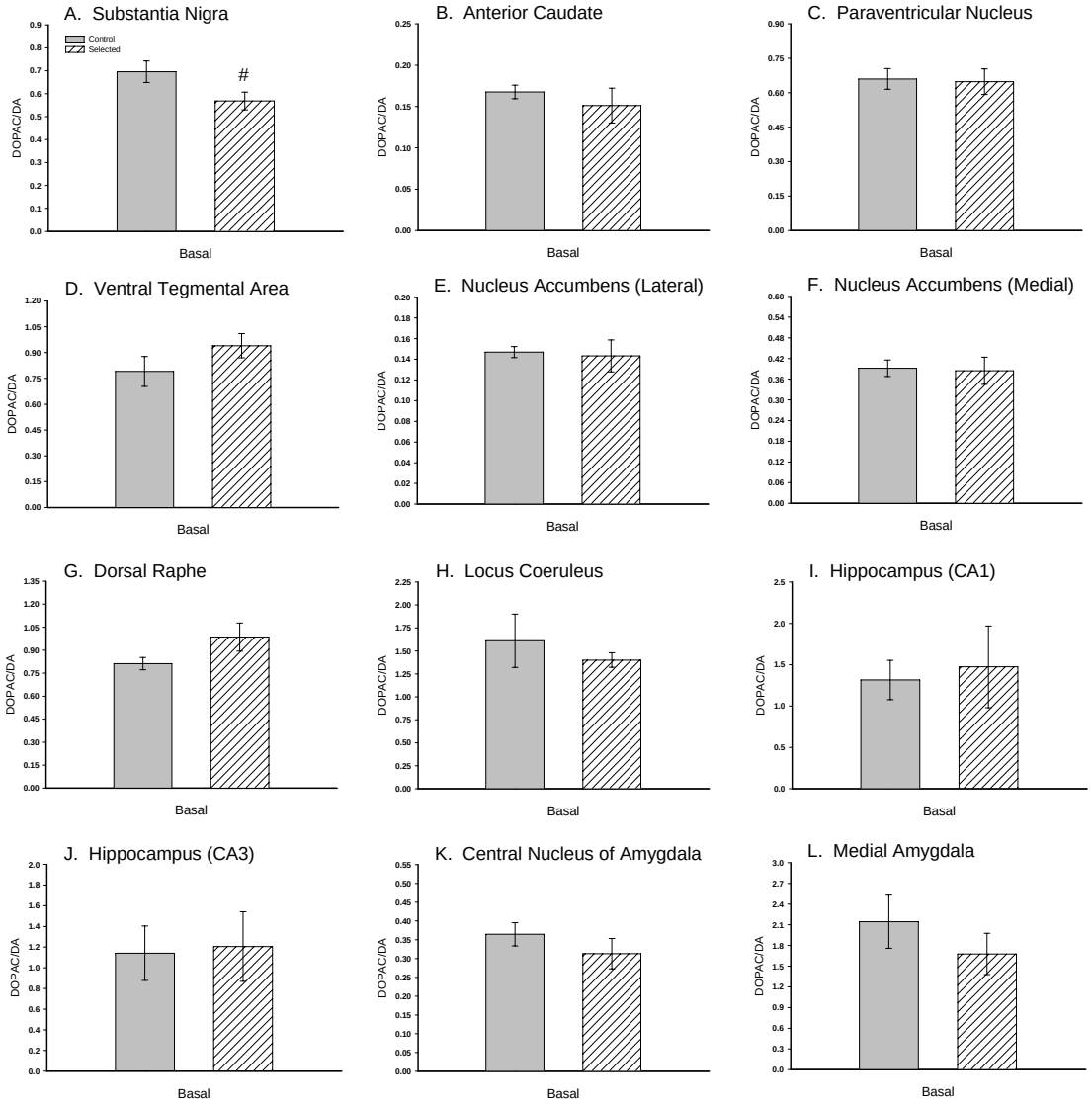

A. Substantia Nigra
Control
Selected
#
DOPAC/DA
Basal
B. Anterior Caudate
C. Paraventricular Nucleus
D. Ventral Tegmental Area
E. Nucleus Accumbens (Lateral)
F. Nucleus Accumbens (Medial)
G. Dorsal Raphe
H. Locus Coeruleus
I. Hippocampus (CA1)
J. Hippocampus (CA3)
K. Central Nucleus of Amygdala
L. Medial Amygdala

Figure 18

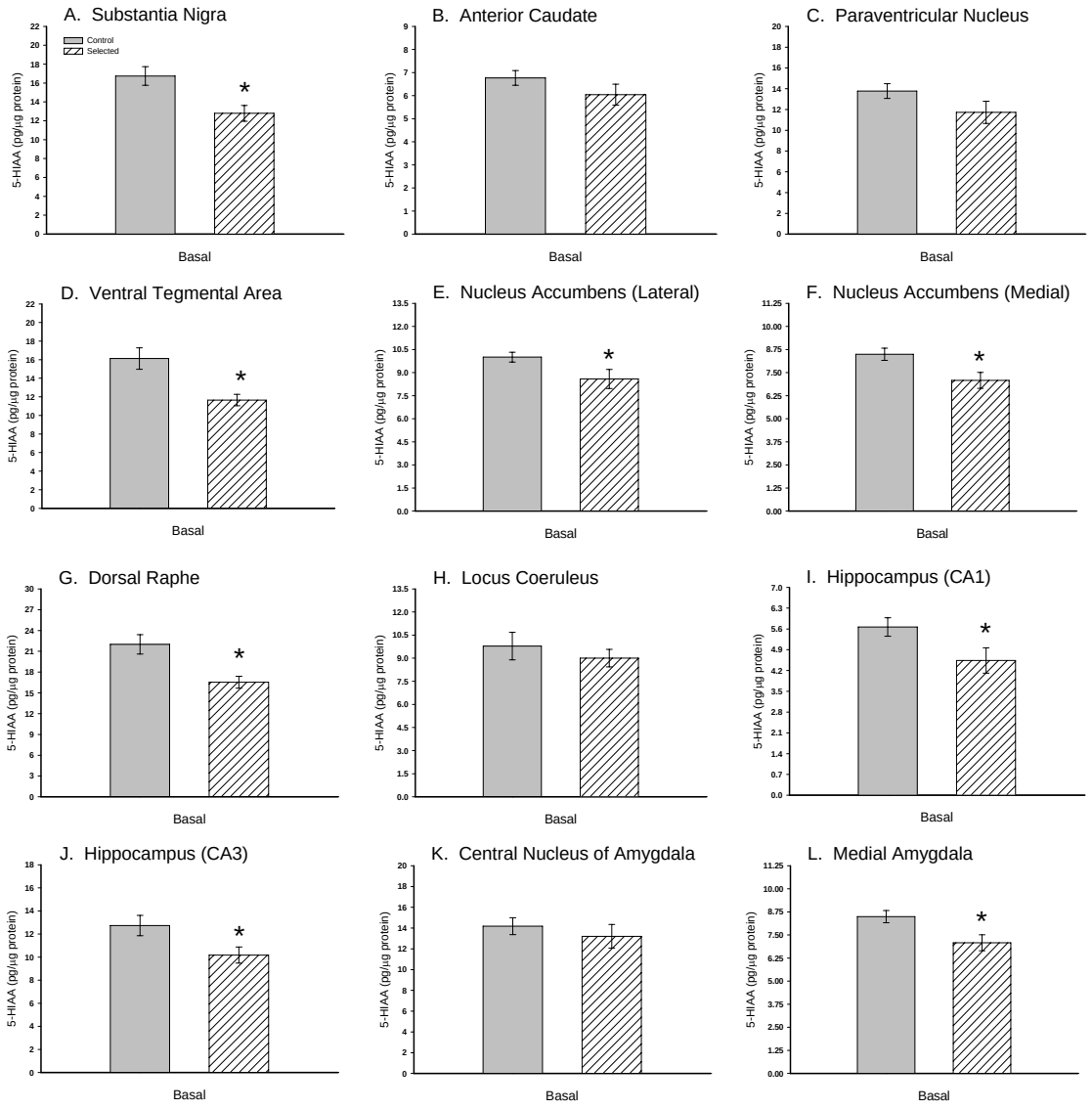

A. Substantia Nigra
B. Anterior Caudate
C. Paraventricular Nucleus
D. Ventral Tegmental Area
E. Nucleus Accumbens (Lateral)
F. Nucleus Accumbens (Medial)
G. Dorsal Raphe
H. Locus Coeruleus
I. Hippocampus (CA1)
J. Hippocampus (CA3)
K. Central Nucleus of Amygdala
L. Medial Amygdala
Control
Selected
5-HIAA (pg/µg protein)
Basal
*

Figure 19

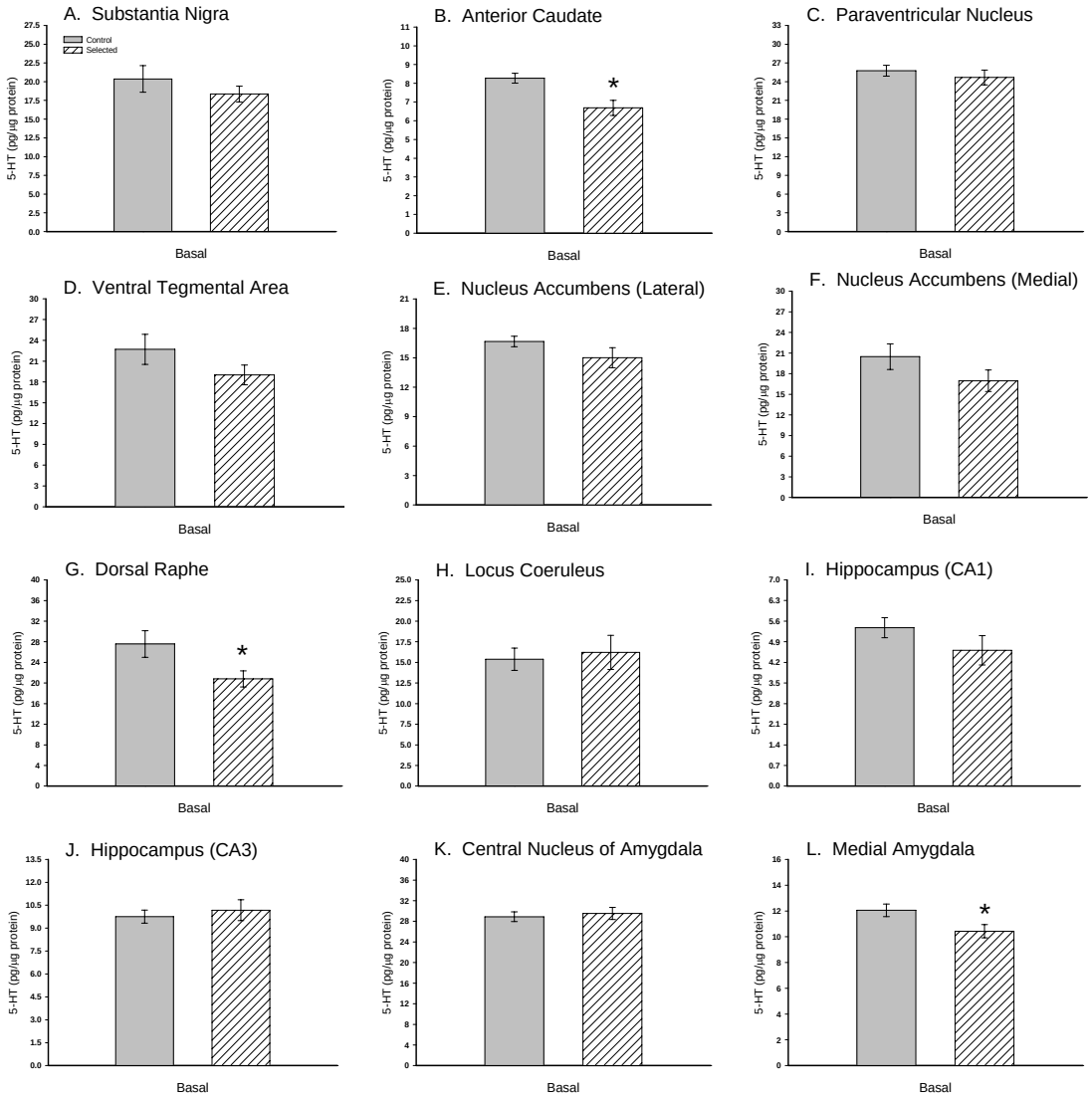
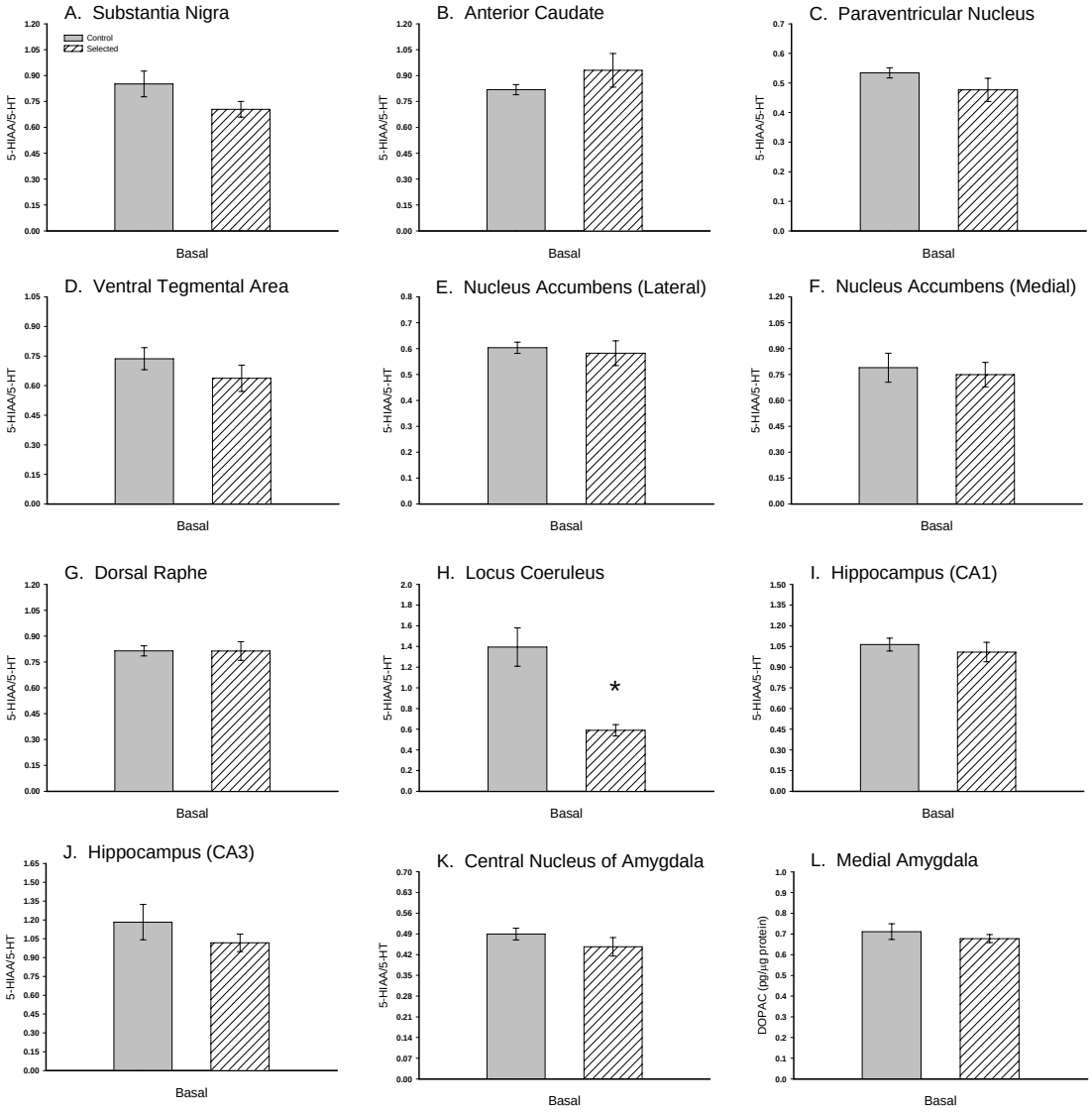

A. Substantia Nigra
Control
Selected
B. Anterior Caudate
C. Paraventricular Nucleus
D. Ventral Tegmental Area
E. Nucleus Accumbens (Lateral)
F. Nucleus Accumbens (Medial)
G. Dorsal Raphe
H. Locus Coeruleus
I. Hippocampus (CA1)
J. Hippocampus (CA3)
K. Central Nucleus of Amygdala
L. Medial Amygdala
5-HT (pg/µg protein)
Basal
*

Figure 20


A. Substantia Nigra
Control
Selected
5-HIAA/5-HT
Basal
B. Anterior Caudate
5-HIAA/5-HT
Basal
C. Paraventricular Nucleus
5-HIAA/5-HT
Basal
D. Ventral Tegmental Area
5-HIAA/5-HT
Basal
E. Nucleus Accumbens (Lateral)
5-HIAA/5-HT
Basal
F. Nucleus Accumbens (Medial)
5-HIAA/5-HT
Basal
G. Dorsal Raphe
5-HIAA/5-HT
Basal
H. Locus Coeruleus
*
5-HIAA/5-HT
Basal
I. Hippocampus (CA1)
5-HIAA/5-HT
Basal
J. Hippocampus (CA3)
5-HIAA/5-HT
Basal
K. Central Nucleus of Amygdala
5-HIAA/5-HT
Basal
L. Medial Amygdala
DOPAC (pg/µg protein)
Basal