Published in final edited form as:

Synapse. 2014 May ; 68(5): 221–233. doi:10.1002/syn.21731.

Characterization of the guinea pig animal model and subsequent comparison of the behavioral effects of selective dopaminergic drugs and methamphetamine

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Abstract

Though not commonly used in behavior tests guinea pigs may offer subtle behavior repertoires that better mimic human activity and warrant study. To test this, 31 Hartley guinea pigs (male, 200–250 g) were evaluated in PhenoTyper cages using the video-tracking EthoVision XT 7.0 software. Results showed that guinea pigs spent more time in the hidden zone (small box in corner of cage) than the food/water zone, or arena zone. Guinea pigs exhibited thigmotaxis (a wall following strategy) and were active throughout the light and dark phases. Eating and drinking occurred throughout the light and dark phases. An injection of 0.25 mg/kg SCH23390, the dopamine D1 receptors (D1R) antagonist, produced significant decreases in time spent in the hidden zone. There were insignificant changes in time spent in the hidden zone for guinea pigs treated with 7.5 mg SKF38393 (D1R agonist), 1.0 mg/kg sulpiride (D2R antagonist), and 1.0 or 10.0 mg/kg methamphetamine. Locomotor activity profiles were unchanged after injections of saline, SKF38393, SCH23390 and sulpiride. By contrast, a single injection or repeated administration for 7 days of low-dose methamphetamine induced transient hyperactivity but this declined to baseline levels over the 22-hour observation period. Guinea pigs treated with high-dose methamphetamine displayed sustained hyperactivity and travelled significantly greater distances over the circadian cycle. Subsequent 7-day treatment with high-dose methamphetamine induced motor sensitization and significant increases in total distances moved relative to single drug injections or saline controls. These results highlight the versatility and unique features of the guinea pig for studying brain-behavior interactions.

Keywords

Locomotor activity; Home-cage occupancy; SCH23390; SKF38393; Sulpiride; EthoVision XT

1. INTRODUCTION

There is a renewed emphasis towards the assessment of animal behavior because it represents the ultimate expression of the interaction between genes, the brain, and the environment. Within this context, mice and rats are commonly used animal models to study behavior in the laboratory for many reasons. The mouse is currently the mammalian model of choice for studying gene–behavior interactions because of its close genetic similarities to humans, and the ease with which its genome can be manipulated and analyzed (Ortiz et al.,

2010; Distler et al., 2012). On the other hand, rats are more social, display sophisticated sexual behaviors, strong spatial navigation and learning and are favored in studying brain-behavior interactions (Berridge, 1990; Whishaw and Tomie, 1996; Crawley and Paylor, 1997; Frick et al., 2000; Kalueff and Tuohimaa, 2005). Altogether, a broad range of standardized tests measuring spontaneous and acquired behaviors, mostly tailored for mice and rats, have been developed. Examples include the open field test (Hall and Ballachey, 1932; Denenberg, 1969) to measure exploratory activity, elevated plus maze (Pellow and File, 1986; Pellow et al., 1985) and light-dark box tests (Crawley and Goodwin, 1980; Crawley, 1985) to measure anxiety-related behaviors and Morris water maze (Morris, 1981) to measure spatial memory. These animal models and behavior tests have proven their usefulness and yielded extensive new knowledge on the genetic and neural regulation of behavior. However, with regard to understanding complex behaviors that are readily translatable to human behaviors or studying human psychiatric disorders like anxiety, mnemonic disorders, and depression, limitations of using mice and rats have been acknowledged (Wahlsten and Rustay, 2006; Tescott and Nestler, 2004; Spruijt and Visser, 2006; Kalueff et al., 2007). For example, an increasing number of studies report that animal attentiveness is a prerequisite for the development of robust visuospatial and olfactory representations in the hippocampus (Muzzio et al., 2009; Rowland et al., 2011). However, it is difficult to measure spontaneous behavior reflective of paying 'attention' towards new objects and environments in both mice and rats (Fantz, 1964; Pascalis and de Haan, 2003; Clark and Martin, 2005; Clark and Squire, 2010; Antunes and Biala, 2011). Consequently, there is a rising need to finding convenient small mammalian animal models for measuring multidimensional behaviors (Ennaceur and Delacour, 1988; Kalueff et al., 2005; Broadbent et al., 2010; Berton et al., 2012; Garcia and Kirkpatrick, 2013).

Though not commonly used in behavior tests, we predicted that guinea pigs may offer subtle behavior repertoires that better mimic human activity and, therefore, warrant study. Guinea pigs are good compact animals that are easy to handle and share many physiological functions with humans (Wagner and Manning, 1976; Terril and Clemons, 1998; Vanderlip, 2003). They are favored animals in ontological research, as the hearing range and structures of the ear are similar to those of humans. Together with primates, the guinea pig is the only laboratory animal with a dietary vitamin C requirement. Guinea pigs are a very good source of serum complement and other hematological parameters (Williams and Festing, 1971); they are widely used in studies of immunology, particularly in studies of delayed hypersensitivity in which its response resembles that of man (Gell and Benacerraf, 1961). Other examples include studies of therapeutic activity spectrum of drugs (Xi and Chase, 2008), anaphylactic shock (Stone et al., 1964), and allergic encephalomyelitis (Takino et al., 1971). Guinea pig brains are a miniature copy of the human brain at many levels including the Circle of Willis which mirrors in detail to that found in humans (cf. Librizzi et al., 1999). Furthermore, mice and rats are considered to be 'altricial' i.e. born at relatively underdeveloped stages with eyes shut and many neurogenic events occur postnatally (Clancy et al., 2007). By contrast, and like humans, guinea pigs are 'precocial' and are born with eyes open with a relatively advanced development of the brain (Verley, 1977; Clancey et al., 2000; Clancey et al., 2007). Thus, the prospects are that guinea pigs may provide a robust small mammalian animal model for measuring multidimensional brain-behavior interactions of direct relevance to human behavior.

In the present study, our major goal was to provide baseline data on the activity of guinea pigs and evaluate how spontaneous behaviors were affected by selective dopaminergic drugs as well as methamphetamine, the mixed monoaminergic stimulant. A related goal, was to determine what other behavioral changes accompany methamphetamine-induced locomotor sensitization thought to reflect drug addiction in animal species. In humans, self-reports of sensitized vigor and energy levels have been described and these behavior manifestations

are presumed to relate to the more direct measurements of locomotor sensitization in animals (Stewart and Badiano, 1993; Robinson and Berridge, 1993; Sax and Strakowski, 2001; Ujike and Sato, 2004). These assumptions have limitations in view of the following. Animal studies primarily focus on locomotor sensitization and few investigators provide information on changes to other behaviors seen in humans. Moreover, animal behavioral tests are typically short lasting and address a single behavior category following stimulant drug challenges. Important information on baseline conditions, circadian rhythms, and interactions between motivational systems are usually not addressed (Tescott and Nestler, 2004; Spruijt and Visser, 2006). Consequently, we know very little about how spontaneous behaviors across circadian cycles are altered by chronic intermittent intake of psychostimulant drugs. Hence, we also proceeded to examine changes to spontaneous and acquired behaviors in the home cage setting that are associated with methamphetamine exposure using our guinea pig animal model.

2. MATERIALS AND METHODS

2.1. Research Animals

Thirty-one male Hartley guinea pigs (outbred; weight 200–250 g) obtained from Charles River Laboratories were housed in pairs without environmental enrichment (i.e. no toys or objects); they had free access to food and water. Guinea pigs were kept on a 12 hr light/dark cycle (lights on at 7:00 AM - 7:00 PM) in the American Association for Laboratory Accreditation Council certified Animal Care Facility (ACF) at Meharry Medical College. The ACF maintains animal rooms at 65–70 °F and 40–70% relative humidity.

2.2. Experiment Groups

After adjustment to the ACF for 7 days, guinea pigs were randomly assigned to six study groups for subsequent treatment with one of the following: (a) phosphate buffered saline, herein denoted as saline), (b) SKF38393, dopamine D1 receptor (D1R) agonist, (c) SCH23390, D1R antagonist, (d) sulpiride, the dopamine D2 receptor (D2R) antagonist, (e) low-dose methamphetamine, the mixed monoaminergic indirect agonist, or (f) high dose methamphetamine. To make stock solutions, 10 mg SCH23390 or 20 mg sulpiride were first dissolved in 0.5 ml dimethyl sulfoxide (DMSO) and then made to final volume by adding 0.5 ml saline, respectively. The stock solutions were subsequently diluted with saline to provide drug concentrations of either 0.25 mg/kg SCH23390 or 1.0 mg/kg sulpiride that were administered to guinea pigs. Thus, the dilution factor for DMSO in the final 1.0 ml injections given to animals ranged between 1:20 and 1:40 for DMSO which corresponds to 0.0125 μ M – 0.025 μ M. We have found that application of these DMSO concentrations do not affect synaptic transmission (data not shown). Methamphetamine, sulpiride, DMSO and phosphate buffered tablets were obtained from Sigma-Aldrich (St. Louis, MO), whereas SCH23390 was acquired from Tocris (Ellisville, MO).

2.3. Activity Assessment in Home Cages

2.3.1. Baseline Activity—Guinea pigs were brought to the research lab while in their ACF home cages and then gently handled by the same experimenter on three consecutive days (i.e. Experiment Days 1–3). During this habituation phase, each guinea pig was placed and left to explore for 5 minutes only in the PhenoTyper multi-purpose observation cage from Noldus (Leesburg, VA). The PhenoTyper cage is constructed of four transparent polymethylmethacrylate (PMMA) walls and a PMMA dual satin opaque cage bottom (dimensions in cm: 45 × 45 × 65) covered with cell-sorb plus bedding made from recycled paper and is odor free (MedNet Direct). The PhenoTyper cage contained food and two water dispensers; it also had a small black shelter (dimensions 15 cm × 15 cm × 12 cm – with entrances on two sides) located in one corner of the cage and this served as a hidden zone

for animals (Figure 1). On the fourth day (Experiment Day-4), guinea pigs were brought back to the research lab between 12:00 noon - 2:00 PM in their ACF home cages. After a short interval, each guinea pig was placed in the PhenoTyper cage and left alone until the next day. During this time guinea pig behavior was recorded unobtrusively using EthoVision XT 7 video-tracking software from Noldus. Guinea pigs had free access to food and water; lights were turned off at 7:00 PM and turned on at 7:00 AM the next day.

2.3.2. Acute Drug Treatment—On Experiment Day-5, guinea pigs were administered via intraperitoneal injections with one of the following: a) 1 ml saline, n=4; b) 7.5 mg/kg SKF38393, n=6; c) 0.25 mg/kg SCH23390, n=6; d) 1 mg/kg sulpiride, n=5; e) 1 mg/kg methamphetamine, n=5; or f) 10 mg/kg methamphetamine, n=5, according to their group. Subsequently each guinea pig was placed back in the PhenoTyper cage for a second day of activity monitoring and recording, i.e. between 2:00 PM until 12:00 noon the following day, which was Experiment Day-6. This marked the end of the baseline activity assessments and guinea pigs were returned to the ACF with the exception of animals in the saline, low-methamphetamine and high methamphetamine groups. These latter animals underwent further study as outlined below.

2.3.3. Chronic Drug Treatment—Guinea pigs from the retained groups were injected with one of the following: a) saline, b) 1.0 mg/kg methamphetamine, or c) 10.0 mg/kg methamphetamine for 7 days. Guinea pigs were then returned to the ACF. In subsequent days (Experiment Days 7–11), guinea pigs were brought to the research laboratory to be injected with the pertinent drugs. Animals were inspected to determine their health status and this included measurement of body weights throughout this treatment phase. After the last drug injection, on Experiment Day-11, each guinea pig was placed back in the PhenoTyper cage and kept there for activity monitoring and recording, i.e. between 2:00 PM until 12:00 noon, until the following day (i.e. Experiment Day-12). This marked the end of the activity assessments and experiments in this series.

2.3.4. Euthanasia—At the completion of experiments guinea pigs were euthanized with an overdose of pentobarbital (150 mg/kg; source: Henry Schein, Melville, NY). All guinea pigs were utilized at Meharry Medical College in accordance with American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals and approved by the Institutional Animal Care and Use Committee.

2.4. Data Analyses

For behavior assessments the EthoVision XT 7 software was used to digitally demarcate the user defined boundaries of the different zones within the PhenoTyper cages. Thus, boundaries were created for the food/water zone, hidden zone and arena zone (i.e. animal not within the hidden or food/water zones; Figure 1B). The EthoVision XT 7 suite was used to create video files of each experiment and subsequently performed in-depth analyses of video-taped animal images off-line. [NB: The number of parameters that can be analyzed is substantial and includes trial duration, time spent in specified zones, distance moved, etc.]. This data was extracted to quantify several parameters including: a) total distances traveled in specific segments of time, b) duration of space occupancy in cage, and c) peak periods of movement.

2.5. Statistical Analysis

Data was analyzed and statistical significance was determined using one-way ANOVA with Tukey's post-hoc test, two-way ANOVA with Bonferroni posthoc test, and/or the paired Student's t-test. Fitness for Gaussian distribution was checked using the D'Agostino and Pearson omnibus normality test (D'Agostino and Pearson, 1973). Variables are reported as

mean $\pm$ SEM unless indicated otherwise ($\alpha=0.05$, and tests were two-sided). The study assessed 31 guinea pigs and at a set significance level of 0.05, this had a statistical power equal to 80% sufficient to detect a 23.2% difference in locomotor activity with a standard deviation of $\pm 44.7\%$. Both statistical and power analyses were performed using GraphPad Prism version 6 and GraphPad StaMate version 2.00 respectively (GraphPad Software, San Diego CA, USA, www.graphpad.com).

3. RESULTS

3.1. Baseline Occupancy Profiles in Home Cage

First, baseline data on cumulative time that guinea pigs spent in different locations were obtained (herein denoted as occupancy) within the PhenoTyper cages before determining how this was affected by the selective dopaminergic drugs and methamphetamine. Thus, occupancy profiles of guinea pigs were unobtrusively video-tracked and analyzed throughout a 22-hour observation period (i.e. from 2:00 PM to 12:00 noon the next day) with lights turned off between 7:00 PM and 7:00 AM (Figure 2A). The results showed that guinea pigs spent $65.6 \pm 1.5\%$ (mean $\pm$ SEM in this and subsequent entries) of their time in the hidden zone with the balance of time spent in the food/water zone ($22.7 \pm 1.7\%$) and open arena ($11.9 \pm 0.8\%$). An analysis of variance showed significant differences in time spent in different cage zones, ($F(2, 78) = 437.7$; $p < 0.0001$). Post-hoc analyses using Tukey's test revealed that the cumulative time spent in the hidden zone was significantly greater than in the food/water zone and arena zone. Furthermore, significantly more time was spent in the food/water zone relative to the arena zone. This occupancy data is summarized in Figure 2B. The results also showed that guinea pigs spent significantly more time in the hidden zone during the light phase ($64.14 \pm 1.38\%$) than they did during the dark phase ($55.68\% \pm 1.47\%$), $t(60) = 4.224$, $p < 0.0001$. A greater proportion of the occupancy around the food/water zone as well as movement in the cage occurred during the dark phase (data not shown). It was noted that extraneous noise disturbance (e.g. loud noises/movements outside room) or when the experimenter entered the room towards the end of the recordings made the guinea pigs 'freeze' in place for long periods or scuttle for cover into the hidden zone. It was clear the hidden zone served as a 'safe zone' where guinea pigs could seek refuge and survey their environment before venturing out to 'play' and feed.

3.2. Baseline Locomotor Activity in Home Cage

Next, locomotor activity in the PhenoTyper cages was evaluated during the same 22-hour observation periods described above. The results showed that guinea pigs remained active throughout the observation period (Figure 3A) and the distance traveled ranged between 14,320 to 90,045 cm (average scores: $43,815 \pm 4,241$ cm; $n = 31$; Figure 3B). Guinea pigs explored the whole open arena space in the cages. However, the majority of the locomotor activity occurred in the peripheral areas of the cage whereas the center had uniformly lower frequency of visits (Figure 3C). Furthermore, animals exhibited 'ultradian' locomotor activity patterns with peak movements occurring around 7:00 PM and secondary peaks taking place at 6–8 hour intervals (Figure 3A and 3B). The results showed that distance moved was significantly greater during the dark phase ($2,529 \pm 198$ cm/hr) relative to the light phase ($1,224 \pm 142$ cm/hr), $t(60) = 5.356$, $p < 0.0001$; Figure 3D). Overall, guinea pigs lacked nocturnal rhythms and there were no indications of protracted intervals reminiscent of prolonged sleep states. Rather eating and drinking occurred throughout the light and dark phases (Figure 2A).

3.3. SCH23390, the D1R Antagonist, Decreases Time Spent in Hidden Zone

After the assessment of spontaneous behaviors was completed guinea pigs were divided into groups and administered single intraperitoneal injections with methamphetamine or one of

the selective-acting dopaminergic drugs. Drug injections, including saline controls, were performed by the same experimenter between 12:00 noon – 2:00 PM each day and these animals were returned in the PhenoTyper cage and their behaviors re-evaluated for another 22-hour period. A one-way ANOVA revealed significant differences between groups in regard to time spent in the hidden zone, $F(5, 132) = 2.533$, $p = 0.0318$ (Figure 4A). Post-hoc comparisons using Tukey's test showed that only guinea pigs treated with 0.25 mg/kg SCH23390 presented with significant reductions in time spent in the hidden zone relative to their saline-injected control animals. Furthermore, significant differences between groups were found in time spent in the food/water zone, $F(5, 132) = 3.343$, $p = 0.0071$ (Figure 4B). Here, Tukey's post-hoc test revealed that guinea pigs treated with SCH23390 or 1.0 mg/kg methamphetamine had significant increases in time spent in the food/water zone relative to animals treated with 1 mg/kg sulpiride. No significant differences were found between the drug groups in comparison to the saline controls in regard to time spent in the arena zone, $F(5, 132) = 1.406$, $p = 0.2262$ (Figure 4C). Taken together treatment with SCH23390 decreased occupancy in the hidden zone perhaps reflecting drug-induced decrease in anxiety levels.

3.4. Methamphetamine Produces Dose-Dependent Changes in Locomotor Activities

Next, locomotor activity levels in one-hour time bins were assessed to determine how these patterns were affected by drug treatments. A summary of the data is shown in Figure 5. The two-factor analysis of variance showed significant differences for drug treatment, $F(5, 575) = 8.256$, $p < 0.0001$, and time elapsed, $F(22, 575) = 4.956$, $p < 0.0001$. The Bonferroni's post hoc test revealed that after low-dose methamphetamine guinea pigs were initially hyperactive and peak locomotor activity was much greater at 7:00 PM. This high level activity was followed by a reduction in locomotor activity for the remainder of the time in the PhenoTyper cage. In the case of high-dose methamphetamine, guinea pigs became hyperactive throughout much of the monitored period but there was an apparent shift for peak activity which now occurred at 4:00 AM instead of 7:00 PM. By comparison, there were insignificant changes to activity levels following saline injections, 7.5 mg/kg SKF38393, the D1R agonist, 0.25 mg/kg SCH23390, the D1R antagonist, or 1.0 mg/kg sulpiride, the D2R antagonist. Again peak activities occurred at 7:00 PM in all these treatment groups. Lastly, the net distances moved by guinea pigs were evaluated after drug injections relative to baseline levels. A one-way analysis of variance revealed significant differences between the treatment groups, $F(5, 132) = 11.39$, $p < 0.0001$. Only guinea pigs treated with high-dose methamphetamine (10 mg/kg) covered significantly more distance over the 22-hour observation period relative to saline controls as well as the other treatment groups (SKF38393, SCH23390, sulpiride, and low-dose methamphetamine; Figure 6).

3.5. Chronic Methamphetamine Alters Occupancy Profiles in Home Cage

It is known that enhancements in locomotor activity termed 'behavioral sensitization' accompany repeated intermittent administration of methamphetamine (Onaivi et al., 2002; McDaid et al., 2006; Hall et al., 2008). However, there is limited information on the changes in animal behaviors across the circadian cycles that are associated with sensitization. To evaluate these behaviors, guinea pigs in the saline group and the two methamphetamine groups were given daily drug injections for 7 days. In each case, guinea pigs were brought to the lab and administered saline or one of the methamphetamine doses between 12:00 noon and 2:00 PM by the same experimenter and then returned back to the ACF. After the last dose, on Day-7, guinea pigs were placed in the PhenoTyper cages and video-tracked for the subsequent 22 hours. One-way analysis of variance showed that the cumulative time spent in the hidden zone differed significantly among the treatment groups, $F(5, 132) = 4.236$, $p = 0.0013$. Tukey's post-hoc comparisons revealed that after a 7-day treatment with low-dose methamphetamine (1 mg/kg) guinea pigs exhibited a significant increase in time spent in the

hidden zone when compared to an acute injection of low-dose methamphetamine and chronically treated with high-dose methamphetamine (10 mg/kg). By contrast, time spent in the hidden zone was not statistically different between acute and chronic treatment with high-dose methamphetamine or saline injections at $p < 0.05$. These results are shown in Figure 7A. Furthermore, a one-way analysis of variance yielded significant differences between groups in regard to time spent in the food/water zone, $F(5, 132) = 2.349$, $p = 0.0444$; Figure 7B. Post-hoc comparisons using Tukey's test revealed that high-dose methamphetamine spent significantly more time in the food/water zone in comparison to guinea pigs that received acute low-dose methamphetamine (Figure 7B). Interestingly, both acute and chronic treatment with high-dose methamphetamine were associated with significant increases in time spent in the arena zone when compared with chronic low-dose methamphetamine, $F(5, 132) = 5.357$, $p = 0.0002$; Figure 7C.

3.6. Chronic Methamphetamine Alters Locomotor Activity Profiles of Guinea Pigs

Chronic methamphetamine was evaluated to determine how guinea pig activity profiles in the PhenoTyper cage were affected. In the case of saline controls, it was found that locomotor activity profiles were unaltered after daily injections for 7 days (Figure 8A). Surprisingly, chronic low-dose methamphetamine (1 mg/kg) no longer produced the initial hyperactivity as was observed following acute injections (Figure 8B). Rather, locomotor activity profile was similar to pre-drug baseline levels. The total distances moved over the 22-hour period were not significantly different relative to pre-drug levels ($p > 0.05$, one-way ANOVA). In the case of chronic high-dose methamphetamine (10 mg/kg) guinea pigs were found to be much more hyperactive than was the case after an acute high-dose methamphetamine. Furthermore, hyperactivity levels were sustained for very prolonged periods that lasted for hours (Figure 8C). Lastly, the total distance moved by guinea pigs after drug injections relative to baseline levels was quantified. A one-way analysis of variance revealed significant differences between the treatment groups, $F(5, 132) = 11.39$, $p < 0.0001$. Guinea pigs treated with high-dose methamphetamine covered significantly more distance over the 22-hour observation period relative to saline controls as well as the other treatment low-dose methamphetamine (Figure 9). These results indicated the presence of behavioral sensitization to drug treatment with high-dose methamphetamine that was still evident in the day long-after exposure to the drug. This was not the case with guinea pigs treated with low-dose methamphetamine, who did not present with significant changes to total distances travelled over the 22 hours period.

4. DISCUSSION

Data presented in this report highlight important activity features exhibited by guinea pigs as a small animal model to assess spontaneous and drug-related behaviors. The study took advantage of computer-based behavioral data acquisition and analysis methods that have expanded the capacity to conduct long-term behavior recordings. We have shown that guinea pigs are very active and lack distinct long period indicative of sleep. Eating and drinking occurs throughout the light phase and dark phase. By contrast, published reports suggest that rats and mice spend about 50% of their time sleeping during the light phase, and they are active during the dark phase (Tobler et al., 1993; Ibuka, 1984). Our findings are consistent with reports in the field indicating that guinea pigs were extraordinarily active and were without alternations between protracted complete rest periods and activity (Nicholls, 1922; Akita et al., 2001; Tobler et al, 1993). For example, Nicholls (1922) found that the guinea pig's time was divided into periods of continuous activity and intermittent activity, whereby, the animals exhibited small rest periods averaging 3–4 minutes in length. Similarly, Tobler and co-workers (1993) conducted EEG recordings over the parietal cortex for 1–2 days and found that sleep in guinea pigs amounted to 32.2%, but sleep patterns were markedly polyphasic and fragmented across the circadian cycle. These published reports,

taken together with our findings, provide important baseline data on guinea pig activity over prolonged periods that will help guide the development of standard and novel experiments probing brain–behavior interactions.

Utilizing our guinea pig model we found that SCH23390 (but not sulpiride, SKF or methamphetamine) decreased the time spent in the hidden zone relative to pre-drug levels. The drug doses we employed were those successfully utilized in previous experiments in the lab (Chirwa et al., 2005; Williams et al., 2006), and that other investigators have found to block late-phase long-term potentiation (late-LTP, a cellular correlate of learning and memory; Bliss and Collingridge, 1993), alter cAMP levels (William et al., 2006), and/or interfere with learning and memory operations (Mehta et al., 2003; Mehta et al., 2008; Lemon et al., 2006). It was unclear from our experiments whether the SCH23390-induced decrease in time spent in the hidden zone reflected a reduction in animal anxiety levels, altered ‘motivations’, or if it was a consequence of hitherto unknown biological effects. Perhaps the SCH23390-associated mediating the reduction in time spent in the hidden zone was partly a consequence of ‘unopposed’ effects of D2Rs signaling after D1Rs were blocked by the drug. Alternatively, SCH23390 may have exerted its effects via its known partial agonistic effects at serotonergic receptors (Briggs et al., 1991; Woodward et al, 1992). More experiments are needed to distinguish between these possibilities. Our study also found that the D1R agonist (SKF38393), or the D1R (SCH23390) and D2R (sulpiride) antagonists did not produce significant changes to total distances travelled relative to pre-drug baseline levels within each animal group and saline controls. In addition, peak locomotor activity still occurred soon after onset of the dark phase in all these treatment groups. Sulpiride is an antipsychotic drug, whereas, SKF38393 and SCH23390 do not currently have clinical indications but are frequently utilized in animal studies that presume unaltered motor activities in the test animals. These assumptions appear to be valid in the doses utilized as shown by our data.

Unlike the selective dopaminergic drugs discussed above, we observed a different activity profile following methamphetamine administration, the indirect mixed monoaminergic agonist. Specifically, treated animals were initially hyperactive following administration of the low-dose (1 mg/kg) and high-dose (10 mg/kg) methamphetamine. However, only guinea pigs treated with high-dose methamphetamine covered significantly greater total distance per day relative to saline controls. Interestingly, even after chronic administration (7-day treatment), animals treated with low-dose methamphetamine were initially hyperactive but the total distance travelled was still unchanged relative to pre-drug levels or saline controls. By contrast, high-dose methamphetamine, when administered as an acute dose and following chronic doses, produced prolonged hyperactivity and significant increases in total distance moved over the 22-hour observation period relative to pre-drug and saline controls. It seemed less likely that the differential effects of methamphetamine were a consequence of higher systemic ‘accumulation’ with the high-dose administration in view of the following. The half-life of methamphetamine is ~12 hours in humans, whereas it is ~70 min in rats (Cho et al., 2001). If we presume a similar drug pharmacokinetic profile in guinea pigs, as occurs in rats, then 99% of any dose of methamphetamine that is given is eliminated in ~7 hours (i.e. exceeds five half-lives for first-order elimination kinetics). We gave single drug injections per day and this meant systemic drug levels decreased to less than 0.01 mg/kg and 0.1 mg/kg within 7 hours for the low- and high-dose methamphetamine, respectively. These levels may not exert much effect on synaptic transmission (cf., Swant et al., 2010). However, it is feasible that while plasma levels of methamphetamine may rapidly decrease, methamphetamine transported into cells by monoamine transporters could attain high intracellular drug concentrations that are dose-dependent and not readily subject to systemic clearance (Volkow et al., 2010) leading to sustained drug effects. Methamphetamine increases the concentration of extracellular dopamine, serotonin, and norepinephrine by

redistributing these neurotransmitters from synaptic vesicles to the cytosol, in addition to inducing reverse transport and competing for transmitter uptake at their cognate transporters (Sulzer et al., 2005; Goodwin et al., 2009). Methamphetamine also affects extracellular glutamate levels (Kaiya et al., 1983; Nash and Yamamoto, 1992; Stephans and Yamamoto, 1994; Swant et al., 2010). Perhaps the involvements of methamphetamine with the various transmitter systems have a dose-dependency and, therefore, account for the differential effects of methamphetamine on activity profiles.

To conclude, we have presented data confirming that guinea pigs share several behavioral characteristics with rats and mice including preference to dark zones and display of thigmotaxic behaviors. Therefore, like rats and mice, guinea pigs would be amenable to experiments involving fear conditioning and/or operant learning paradigms. Additionally, guinea pigs stand out in other key areas such as being non-nocturnal animals and exhibiting polyphasic, fragmented sleep patterns (Ibuka, 1984; Tobler et al., 1993; Xi and Chase, 2008). Firstly, guinea pigs are voracious eaters (Nicholls, 1922) which make them ideal in studies utilizing food as a reward in operant condition. The advantage is that prior reduction in access to food may not be necessary to optimize experiments. In general the eating behaviors of guinea pigs may be a useful model for understanding eating disorders in humans. Secondly, when performing behavior experiments, it is important for the testing times to match the animal's arousal state. Guinea pigs are neither nocturnal nor diurnal (Nicholls, 1922; Tobler et al., 1993) and, therefore, do not require reversal of light–dark cycles often necessary when using mice or rats (Prager et al., 2011). Thirdly, guinea pigs continuous and intermittent activity levels offer unique opportunities for investigating the impact of sleep patterns on various physiological processes. For example, their apparent polyphasic and fragmented sleep patterns, distributed throughout a circadian cycle offer unique opportunity for assessing the role of sleep in complex brain operations such as memory encoding and consolidation. Another example, humans exhibit circadian rhythmicity of core body temperature, and of growth hormone and cortisol levels in the blood. How these processes are linked to the sleep–wake cycle timings is unclear and the guinea pig model can be useful in providing answers to these outstanding questions. Lastly, the common view of guinea pigs being docile and/or freezing in place is misleading and perhaps fails to capture the importance of this quantifiable response. This is because most behavioral experiments are performed in restricted new environments and for short durations, addressing a single behavioral response. The long-term effects of human handling and/or circadian processes that drive behavior to a great extent are omitted (Tescott and Nestler, 2004; Spruijt and Visser, 2006). Failure to consider these factors invariably diminishes accurate assessment of the significance of an observed behavioral response in relation to the organism's interaction with its environment as part of its survival strategies. The present study took this into account and confirms that guinea pigs are very active animals (Nicholls, 1922; Akita et al., 2001) and form a robust small animal model for measuring brain–behavior interactions.

Acknowledgments

The authors wish to thank Drs. Aramandla Ramesh and Twum Ansah for their helpful review and feedback to an earlier version of the manuscript. The manuscript was written and approved by all the authors. There are no conflicts of interest associated with the reported work.

This work was supported by National Institutes of Health research grant DA021471 and training grant NS061201.

References

- Akita M, Ishii K, Kuwahara M, Tsubone H. The daily pattern of heart rate, body temperature, and locomotor activity in guinea pigs. *Exp Anim*. 2001; 50:409–415. [PubMed: 11769543]

- Antunes M, Biala G. The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cognit Process*. 2011; 13:93–110. [PubMed: 22160349]
- Bell DS. The experimental reproduction of amphetamine psychosis. *Arch Gen Psych*. 1973; 29:35–40.
- Berridge KC. Comparative fine structure of action: rules of form and sequence in the grooming patterns of six rodent species. *Behavior*. 1990; 113:21–56.
- Berton O, Hahn C-G, Thase ME. Are we getting closer to valid translational models for major depression? *Science*. 2012; 338:75–79. [PubMed: 23042886]
- Bliss TVP, Collingridge G. A synaptic model of memory and long-term potentiation in the hippocampus. *Nature*. 1993; 361:31–39. [PubMed: 8421494]
- Briggs CA, Pollock NJ, Frail DE, Paxson CL, Rakowski RF, Kang CH, Kebabian JW. Activation of the 5-HT_{1C} receptor expressed in *Xenopus* oocytes by the benzazepines SCH 23390 and SKF 38393. *Br J Pharmacol*. 1991; 104:1038–1044. [PubMed: 1687364]
- Broadbent NJ, Gaskin S, Squire LR, Clark RE. Object recognition memory and the rodent hippocampus. *Learn Mem*. 2010; 17:5–11. [PubMed: 20028732]
- Chirwa S, Aduonum A, Pizarro J, Reasor J, Kawai Y, Gonzalez M, McAdory B, Onaivi E, Barea-Rodriguez EJ. Dopaminergic DA1 signaling couples growth-associated protein-43 and long term potentiation in guinea pig hippocampus. *Brain Res Bull*. 2005; 64:433–440. [PubMed: 15607831]
- Cho AK, Melega WP, Kuczenski R, Segal DS. Relevance of pharmacokinetic parameters in animal models of methamphetamine abuse. *Synapse*. 2001; 39:161–166. [PubMed: 11180503]
- Clancey B, Darlington RB, Finlay BL. The course of human events: predicting the timing of primate neural development. *Developmental Science*. 200; 3:57–66.
- Clancy B, Finlay BL, Darlington RB, Anand KJS. Extrapolating brain development from experimental species to humans. *Neurotox*. 2007; 28:931–937.
- Clark RE, Martin SJ. Interrogating rodents regarding their object and spatial memory. *Current Opin Neurobiol*. 2005; 15:593–598.
- Clark RE, Squire LR. An animal model of recognition memory and medial temporal lobe amnesia: History and current issues. *Neuropsychologia*. 2010; 48:2234–2244. [PubMed: 20144894]
- Crawley JN, Goodwin FN. Preliminary report of a simple animal behavior for the anxiolytic effects of benzodiazepines. *Pharmacol Biochem Behav*. 1980; 13:167–170. [PubMed: 6106204]
- Crawley JN, Paylor R. A proposed test battery and constellations of specific behavioral paradigms to investigate the behavioral phenotypes of transgenic and knockout mice. *Horm Behav*. 1997; 31:197–211. [PubMed: 9213134]
- Crawley JN. Exploratory behavior models of anxiety in mice. *Neurosci Biobehav Rev*. 1985; 9:37–44. [PubMed: 2858080]
- D'Agostino R, Pearson ES. Tests for departure from Normality: Empirical results for the distributions of b_2 and b_1 . *Biometrika*. 1973; 60:613–622.
- Denenberg VH. Open-field Behavior in the Rat: What Does it Mean? *Ann New York Acad Sci*. 1969; 159:852–859. [PubMed: 5260302]
- Distler MG, Opal MD, Dulawa SC, Palmer AA. Assessment of behaviors modeling aspects of schizophrenia in *csmd1* mutant mice. *PLoS One*. 2012; 7(12):e51235.10.1371/journal.pone.0051235 [PubMed: 23284669]
- Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats I: behavioral data. *Behav Brain Res*. 1988; 31:47–59. [PubMed: 3228475]
- Fantaz RL. Visual experience in infants: Decreased attention to familiar patterns relative to novel ones. *Science*. 1964; 146:668–670. [PubMed: 14191712]
- Frick KM, Stillner ET, Berger-Sweeney J. Mice are not little rats: species differences in a one-day water maze task. *Neuroreport*. 2000; 11:3461–3465. [PubMed: 11095500]
- Garcia A, Kirkpatrick K. Impulsive choice behavior in four strains of rats: Evaluation of possible models of attention-deficit/hyperactivity disorder. *Behav Brain Res*. 2013; 238:10–22. [PubMed: 23085479]
- Gell, PGH.; Benacerraf, B. Delayed hypersensitivity to simple proteins. In: Taliaferro, WH.; Humphrey, JH., editors. *Advances in Immunology*. Vol. 1. New York: Academic Press; 1961. p. 319-343.

- Goodwin JS, Larson GA, Swant J, Sen N, Javitch JA, Zahniser NR, De Felice LJ, Khoshbouei H. Amphetamine and methamphetamine differentially affect dopamine transporters in vitro and in vivo. *J Biol Chem*. 2009; 284:2978–2989. [PubMed: 19047053]
- Hall CS, Ballachey EL. A study of the rat's behavior in a field: a contribution to method in comparative psychology. University of California Publications in Psychology. 1932; 6:1–12.
- Hall DA, Stanis JJ, Avila HM, Gulley JM. A comparison of amphetamine- and methamphetamine-induced locomotor activity in rats: evidence for qualitative differences in behavior. *Psychopharm*. 2008; 195:469–478.
- Ibuka N. Ontogenesis of circadian sleep-wakefulness rhythms and developmental changes of sleep in the altricial rat and in the precocial guinea pig. *Behav Brain Res*. 1984; 11:185–196. [PubMed: 6721913]
- Kalueff AV, Minasyan A, Tuohimaa P. Behavioral characterization in rats using the elevated alley Suok test. *Behav Brain Res*. 2005; 165:52–57. [PubMed: 16144722]
- Kalueff AV, Tuohimaa P. The grooming analysis algorithm discriminates between different levels of anxiety in rats: potential utility for neurobehavioral stress. *J Neurosci Meth*. 2005; 143:169–177.
- Kalueff AV, Wheaton M, Murphy DL. What's wrong with my mouse model? Advances and strategies in animal modeling of anxiety and depression. *Behav Brain Res*. 2007; 179:1–18. [PubMed: 17306892]
- Kaiya H, Takeuchi K, Yoshida H, Kondo T, Sanpei F, Okada Y, Namba M. Effects of subchronic treatment of methamphetamine haloperidol on the rat brain levels of GABA, glutamate and aspartate. *Folia Psychiatr Neurol Japan*. 1983; 37:107–113.
- Lemon N, Manahan-Vaughan D. Dopamine D1/D5 receptors gate the acquisition of Novel Information through Hippocampal long term potentiation and long term depression. *J Neurosci*. 2006; 26:7723–7729. [PubMed: 16855100]
- Librizzi L, Biella G, Cimino C, De Curtis M. Arterial supply of limbic structures in the guinea pig. *J Comp Neurol*. 1999; 411:674–682. [PubMed: 10421876]
- Logan BK. Methamphetamine and driving impairment. *J Forensic Sci*. 1996; 41:457–464. [PubMed: 8656187]
- McDaid J, Graham MP, Napier TC. Methamphetamine-induced sensitization differentially alters pCREB and deltaFosB throughout the limbic circuit of the mammalian brain. *Molec Pharmacol*. 2006; 70:2064–2074. [PubMed: 16951039]
- Mehta MA, McGowan SW, Lawrence AD, Aitken MR, Montgomery AJ, Grasby PM. Systemic sulphuride modulates striatal blood flow: relationships to spatial working memory and planning. *Neuroimage*. 2003; 20:1982–1994. [PubMed: 14683704]
- Mehta MA, Montgomery AJ, Kitamura Y, Grasby PM. Dopamine D2 receptor occupancy levels of acute sulphuride challenges that produce working memory and learning impairments in healthy volunteers. *Psychopharmacology (Berlin)*. 2008; 196:157–165. [PubMed: 17912501]
- Morris RGM. Spatial localization does not require the presence of local cues. *Learning & Motiv*. 1981; 12:239–260.
- Muzzio IA, Levita L, Kulkarni J, Monaco J, Kentros C, Stead M, Abbott LF, Kandel ER. Attention enhances the retrieval and stability of visuospatial and olfactory representations in the dorsal hippocampus. *Plos Biol*. 2009; 7(1–20):e1000140. [PubMed: 19564903]
- Nash JF, Yamamoto BK. Methamphetamine neurotoxicity and striatal glutamate release: comparison to 3,4-methylenedioxymethamphetamine. *Brain Res*. 1992; 581:237–243. [PubMed: 1356579]
- Nicholls E. A study of the spontaneous activity of the guinea pig. *Comp Psych*. 1922; 2:303–330.
- Onaivi ES, Ali SF, Chirwa SS, Zwiller J, Thiriet N, Akinshola BE, Ishiguro H. Ibogaine signals addiction genes and methamphetamine alteration of long-term potentiation. *Annals New York Acad Sci*. 2002; 965:24–86.
- Ortiz O, Delgado-García JM, Espadas I, Bahí A, Trullas R, Dreyer JL, Gruart A, Moratalla R. Associative Learning and CA3–CA1 Synaptic Plasticity Are Impaired in D1R Null, Drd1a/ Mice and in Hippocampal siRNA Silenced Drd1a Mice. *J Neurosci*. 2010; 30:12288–12300. [PubMed: 20844125]

- Pascalis, O.; de Haan, M. Recognition memory and novelty preference: What model?. In: Hayne, H.; Fagen, J., editors. *Progress in infancy research*. Vol. 3. Lawrence Erlbaum Associates; New Jersey: 2003. p. 95-120.
- Pellow S, File SE. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: A novel test of anxiety in the rat. *Pharmacol Biochem Behav*. 1986; 24:525–529. [PubMed: 2871560]
- Pellow S, Chopin P, File SE, Briley M. Validation of open closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods*. 1985; 14:149–167. [PubMed: 2864480]
- Prager EM, Bergstrom HC, Grunberg NE, Johnson LR. The importance of reporting housing and husbandry in rat research. *Front Behav Neurosci*. 2011; 5:38.10.3389/fnbeh.2011.00038 [PubMed: 21847375]
- Robinson TE, Berridge KC. The neural basis of drug craving: an incentive sensitization theory of addiction. *Brain Res Rev*. 1993; 18:247–291. [PubMed: 8401595]
- Rowland DC, Yanovich Y, Kentros CG. A stable hippocampal representation of a space requires its direct experience. *PNAS*. 2011; 108:14654–14658. [PubMed: 21852575]
- Sax KW, Strakowski SM. Behavioral sensitization in humans. *J Addict Dis*. 2001; 20:55–65. [PubMed: 11681593]
- Spuijdt BM, Visser de L. Advanced behavioral screening: Automated home cage ethology. *Drug discovery today Technol*. 2006; 3:231–237.
- Stephans SE, Yamamoto BK. Methamphetamine-induced neurotoxicity: roles for glutamate and dopamine efflux. *Synapse*. 1994; 17:203–209. [PubMed: 7974204]
- Stone SH, Liacopoulos P, Liacopoulos-Briot M, Neven T, Halpern BN. Histamine differences in amount available for release in lungs of guinea pigs, susceptible and resistant to acute anaphylaxis. *Science*. 1964; 146:1061–1062. [PubMed: 17832406]
- Stewart J, Badiani A. Tolerance and sensitization to the behavioral effects of drugs. *Behav Pharmacol*. 1993; 4:289–312. [PubMed: 11224198]
- Sulzer D, Sonders MS, Poulsen NW, Galli A. Mechanisms of neurotransmitter release by amphetamines: a review. *Prog Neurobiol*. 2005; 75:406–433. [PubMed: 15955613]
- Swant J, Chirwa S, Stanwood G, Khoshbouei H. Methamphetamine reduces LTP and increases baseline synaptic transmission in the CA1 region of mouse hippocampus. *PLoS ONE*. 2010; 5(6):e11382. [PubMed: 20614033]
- Takino Y, Sugahara K, Horino I. Two lines of guinea pigs sensitive and non-sensitive to chemical mediators and anaphylaxis. *J Allergy*. 1971; 47:247–261. [PubMed: 5280451]
- Terril, LA.; Clemons, DJ. The laboratory guinea pig. In: Suckow, MA., editor. *The laboratory animal pocket reference series*. CRC Press; 1998.
- Tescott LH, Nestler EJ. Neurobehavioral assessment in the information age. *Nature Neurosci*. 2004; 7:462–466. [PubMed: 15114359]
- Tobler I, Franken P, Jaggi. Vigilance states, EEG spectra, and cortical temperature in the guinea pig. *Am J Physiol Regulatory Integrative Comp Physiol*. 1993; 264:1125–1132.
- Ujike H, Mitsumoto Sato. Clinical Features of Sensitization to Methamphetamine Observed in Patients with Methamphetamine Dependence and Psychosis. *Ann NY Acad Sci*. 2004; 1025:279–287. [PubMed: 15542728]
- Vanderlip, S. *The guinea pig handbook*. Barron's Education Series Inc; 2003.
- Verley R. Comparative development of the mammalian nervous system. *Rev Electroencephalogr Neurophysiol Clin*. 1977; 7:245–254. [PubMed: 412238]
- Volkow ND, Fowler JS, Wang G-J, Shumay E, Telang F, Thanos PK, Alexoff D. Distribution and pharmacokinetics of methamphetamine in the human body: clinical implications. *PLoS ONE*. 2010; 5(12):e15269. [PubMed: 21151866]
- Wagner, JE.; Manning, PJ. *The biology of the guinea pig*. Academic Press; 1976.
- Wahlsten D, Rustay NR. In search for a better mouse test. *TINS*. 2003; 2006:132–136. [PubMed: 12591215]
- Whishaw IQ, Tomie IA. Of mice and mazes: similarities between mice and rats on dry land but not water mazes. *Physiol Behav*. 1996; 60:1191–1197. [PubMed: 8916170]

- Williams J, Festing MFW. A note on some hematological parameters in three strains of guinea pigs. *Guineapig Newsletter*. 1971; 2:7–11.
- Williams S, Mmbaga N, Chirwa S. Dopaminergic D1 receptor agonist SKF 38393 induces GAP-43 expression and long term potentiation in hippocampus in vivo. *Neurosci Lett*. 2006; 402:46–50. [PubMed: 16675111]
- Woodward RM, Panicker MM, Miledi R. Actions of dopamine and dopaminergic drugs on cloned serotonin receptors expressed in *Xenopus* oocytes. *Neurobiol*. 1992; 89:4708–4712.
- Xi M, Chase MH. Effects of eszopiclone and zolpidem on sleep and waking states in the adult guinea pig. *Sleep*. 2008; 31:1043–1051. [PubMed: 18652100]

Figure 1.

(A) The customized PhenoTyper cage from Noldus was used to assess guinea pig behaviors. The basic components of the cage included a feeder, water bottles, and small shelter in one corner of the cage that was the hidden zone. The cage walls were covered with white construction paper to create contrast with the floor bedding, which was grey. The construction paper was raised 1.5–2 inches from the floor bedding to allow the guinea pig to see their home cage mate in the adjacent PhenoTyper cage. The top cover of the cage housed a CCD black/white camera that detected infrared light up to ~980 nm. The camera was equipped with a high pass infrared filter that blocked light in the visible range and, therefore, recording occurred independent of visible light. (B) Top view of the cage floor showing the different zones digitally demarcated using EthoVison XT 7.0 software. Automated video-tracking could be conducted continuously unobtrusively and without the experimenter in the room.

Figure 2.

(A) Representative occupancy data for one guinea pig throughout the light and dark phases. The black horizontal bar along the X-axis corresponds to time when lights were switched off from 7:00 PM to 7:00 AM the next day. Each plotted vertical bar represents a 1-hour segment and the Y-axis denotes percentage of time of the hour segment that the guinea pig spent in each zone. (B) Summary of group data ($n = 31$ guinea pigs) showing cumulative times spent in the three zones namely the hidden zone, food/water and arena zones. There were significant differences in occupancy times among the different zones. Asterisks indicate significant differences among the different zones, $F(2, 78)=437.7$; $p < 0.0001$; one-way ANOVA with Tukey's post-hoc test.

Figure 3.

(A) A representative baseline activity profile recorded for a guinea pig inside the PhenoTyper cage during a 22-hour period (same guinea pig shown in Figure 2). Each plotted vertical bar represents a 1-hour segment and the Y-axis reflects the percentage of time scored as moving (> 6 cm/sec; red) or not moving (< 6 cm/sec; grey). The black horizontal bar along the X-axis corresponds to the dark phase between 7:00 PM to 7:00 AM the next day. Guinea pigs were found to be active throughout the light- and dark-phase. (B) Group data showing guinea pig baseline locomotor activities continuously tracked in the PhenoTyper cages during the 22-hour monitored period. Guinea pigs exhibited crepuscular behavior with peak locomotor activities occurring at 7:00 PM under our experimental conditions. (C) Sample tracing of guinea pig locomotor activity observed during peak activity at 7:00 PM. (D) Summary of group data showing significant differences in total distances traveled in light phase versus dark phase. Asterisks indicates significant difference, $t(60) = 5.356$, $p < 0.0001$; $n = 31$ guinea pigs.

Figure 4.

After characterizing baseline spontaneous behaviors, guinea pigs were divided into groups of 5–6 animals and treated with selective dopaminergic drugs or methamphetamine, the mixed indirect monoaminergic agonist. Guinea pig behaviors were then video-tracked for the next 22-hour period. The data are presented as group histograms showing the net differences in occupancies in the different zones following drug treatments. (A) Data shows that only guinea pigs treated with the D1R antagonist, SCH23390, presented with significant decreases in time spent in the hidden zone relative to saline controls, $F(5, 132) = 2.533$; $p = 0.0318$. By contrast there were insignificant changes to time spent in the food/water zones (B), or arena zone (C) for the SKF 38393, sulpiride, low- and high-dose methamphetamine groups relative to the saline controls. There was a significant increase in time spent in the food/water zone for guinea pigs treated with SCH23390 or low-dose methamphetamine when compared to animals treated with the D2R antagonist, sulpiride, $F(5, 132) = 3.343$, $p < 0.0071$. Asterisks indicate significant differences as determined with one-way ANOVA and Tukey's post-hoc test.

Figure 5.

Group data of distances travelled by guinea pigs in the PhenoTyper cage before (●) and after (■) drug treatments ($n = 5-6$ guinea pigs per group). Plotted values are distances moved in each hour reported as mean $\pm$ SEM ($n = 4-6$ per group). Activity profiles were unaltered after saline (A), SKF38393 (B), SCH23390 (C), or sulpiride (D), and peak activities still occurred at 7:00 PM in these animal groups (A-D). Low-dose methamphetamine guinea pigs exhibited a trend towards more pronounced and longer lasting peak locomotor activity during the 7:00 PM interval (E). However, this change was not significantly different from pre-drug levels in this group. This was followed by return of locomotor activity to baseline levels. High-dose methamphetamine guinea pigs presented with significant increases in activity throughout the monitored period and there was an apparent shift for peak activity which now occurred at 4:00 AM instead of 7:00 PM relative to pre-drug levels (Time Factor: $F(22, 176) = 1.693$, $p < 0.05$, repeated measures 2-way ANOVA with Bonferroni post-hoc test).

Figure 6.

Average changes in distances travelled by guinea pigs in the PhenoTyper cage following drug treatments over the 22-hour period. The histograms are derived from taking the net difference in distance traveled after drug treatment minus pre-treatment values in each group, respectively. Data shows that only guinea pigs treated with 10 mg/kg methamphetamine presented with significant increases in locomotor activity relative to saline controls and other treatment groups, whereas the values for the other groups were unchanged ($F(5,132) = 11.39$, $p < 0.0001$; one-way ANOVA with Tukeys post-hoc test).

Figure 7.

Net changes in cumulative time spent in different zones in PhenoTyper cage after 7-day (chronic) drug treatments. (A) Time spent in the hidden zone was statistically different between chronic treatment with low-dose methamphetamine and acute low methamphetamine and chronic high-dose methamphetamine, $F(5, 132) = 4.236$, $p = 0.0013$. (B) High-dose methamphetamine spent significantly more time in the food/water zone in comparison to guinea pigs that received acute low-dose methamphetamine, $F(5, 132) = 2.349$, $p = 0.0444$. (C) Both acute and chronic treatment with high-dose methamphetamine were associated with significant increases in time spent in the arena zone when compared with chronic low-dose methamphetamine and saline controls, $F(5, 132) = 5.357$, $p = 0.0002$. Statistical tests are one-way ANOVA with Tukey's post-hoc tests. Abbreviations: SAa, saline, acute; SAC, saline, chronic; LMa, low-dose methamphetamine, acute; LMC, low-dose

methamphetamine, chronic; HMa, high-dose methamphetamine, acute; HMc, high-dose methamphetamine, chronic.

Figure 8.

Guinea pig activity profiles after chronic administration of methamphetamine. (A) Locomotor activity profiles were unaltered in saline controls. Peak activity still occurred at 7:00 PM. Each point represents mean $\pm$ SEM in all the plots, and $n = 4$ per group). (B) Animals exhibited initial hyperactivity with pronounced locomotor activity at 7:00 PM following chronic low-dose methamphetamine. Chronic low-dose methamphetamine produced the same effects in guinea pigs as seen with acute low-dose methamphetamine and baseline. (C) In the case of chronic high-dose methamphetamine guinea pigs were found to be much more hyperactive than was the case after an acute high-dose methamphetamine.

Furthermore, hyperactivity levels were sustained for very prolonged periods as shown in figure.

Figure 9.

Changes in total distance traveled after chronic drug treatments. Guinea pigs treated with both acute or chronic high-dose methamphetamine covered significantly more distance over the 22-hour observation period relative to saline controls or low-dose methamphetamine, $F(5, 132) = 11.39$, $p < 0.0001$, one-way ANOVA with Tukey's post-hoc test.