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Overexpression of Protein Kinase Inhibitor Alpha Reverses Rat Low Voluntary Running Behavior

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Abstract

A gene was sought that could reverse low voluntary running distances in a model of low voluntary wheel-running behavior. In order to confirm the low motivation to wheel-run in our model does not result from defects in reward valuation, we employed sucrose preference and conditioned place preference for voluntary wheel-access. We observed no differences between our model and wild-type rats regarding the aforementioned behavioral testing. Instead, low voluntary runners seemed to require less running to obtain similar rewards for low voluntary running levels compared to wild-type rats. Previous work in our lab identified protein kinase inhibitor alpha as being lower in low voluntary running than wild-type rats. Next, nucleus accumbens injections of an adenoviral-associated virus that overexpressed the protein kinase inhibitor alpha gene increased running distance in low voluntary running, but not wild-type rats. Endogenous mRNA levels for protein kinase inhibitor alpha, dopamine receptor D1, dopamine receptor D2, and Fos were all only lower in wild-type rats following overexpression compared to low voluntary runners, suggesting a potential molecular and behavioral resistance in wild-type rats. Utilizing a nucleus accumbens preparation, three intermediate early gene mRNAs increased in low voluntary running slices after dopamine receptor agonist SKF-38393 exposure, while wild-type had no response. In summary, the results suggest that protein kinase inhibitor alpha is a promising gene candidate to partially rescue physical activity in the polygenic model of low voluntary running. Importantly, there were divergent molecular responses to protein kinase inhibitor alpha overexpression in low voluntary runners compared to wild-type rats.

Keywords Behavior · Gene · Brain · Rescue · Voluntary running · Selective breeding

Introduction

Experts have stated that "efforts are desperately needed throughout the health care system in the United States, and throughout the world, to increase physical activity levels" [1]. The extent of the problem being that ~ 97% of adult, US citizens fail to meet US guidelines to obtain 30 min a day of physical activity [2]. A staggering reality considering that lifetime physical inactivity is associated with 40 known chronic diseases and conditions [3]. Two recent findings suggest the existence of genetic factors for the low motivation to be physically active. Den Hoed et al. [4, 5] determined that human sedentary behavior is 31% heritable, and Roberts et al. [5–7] developed a selectively bred rat model of low voluntary wheel-running (LVR) behavior, both of which suggest the existence of genetic determinants for inactivity behavior.

Though many neuronal processes behind wheel-running behavior and physical activity motivation still remain elusive, several lines of evidence suggest the likely role of mesolimbic reward pathway in influencing the motivation to be physically active [8, 9]. More explicitly, motivation has been linked to the nucleus accumbens (NAc), a forebrain structure thought to translate "motivation into goal directed behaviors" [10]. Largely, this has been attributed to dopamine acting at the

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level of the NAc to increase the reinforcing properties of a behavior over time, such as long-term wheel-running [9, 11]. Reward-incentive motivation has been associated with molecular and cellular changes in the NAc [12, 13]. In so, dopamine-signaling acting through D1-like receptors in the NAc is known to initiate a cascade of intracellular events, and in particular, the production of cyclic adenosine monophosphate (cAMP) and activation of cAMP-dependent protein kinase (PKA) [14, 15], which have been shown to influence motivational behaviors, such as long-term wheel-running in rodents [16].

Our preliminary studies of RNA sequencing of the NAc revealed a lower mRNA expression of the PKIα (Protein Kinase Inhibitor alpha) isoform in LVR compared to high voluntary wheel-running (HVR) rats [17]. We also observed a strong positive correlation between PKIα mRNA expression and nightly running distance and time, independent of our selection line [17]. PKIα is a strong inhibitor of protein kinase A (PKA). Proper regulation of PKA is primarily accomplished via two cellular mechanisms: (1) the inhibition of either the catalytic or the regulatory subunits of PKA, and (2) the regulation of the intracellular localization of the free catalytic subunits of PKA [18]. The protein kinase inhibitor (PKI) family meets both PKA regulation criteria by means of its interactions with both functional PKA domains [18–20]. Given the critical position of endogenous protein kinase inhibitors, such as the PKIα isoform, to regulate reward and motivational signaling events, this family of molecules has the potential to function as a molecular driver of physical activity motivation acting through the PKA pathway.

In so, we hypothesized the decreased PKIα expression in the NAc of LVR rats accounts for a decreased break on dopamine signaling, possibly implying that less wheel-running is needed to garner the desired reward from running in these animals. The goals of the current study were to (1) determine expression difference in PKIα expression between LVR and wild-type (WT) rats; (2) address whether rats selectively bred to run low nightly distances experience reward-incentive motivation concerning long-term wheel-running; (3) evaluate the role of PKIα in modulating molecular markers commonly associated with reward and motivation, in hopes of elucidating the potential actions of this molecule in altering wheel-running motivation; and (4) determine the extent to which modifying PKA activity, both pharmacologically and via the overexpression of PKIα in the NAc of WT and LVR lines, increases wheel-running behavior, and, by extension, the motivation to be physically active. Herein, we reveal that local, NAc supplementation of the gene PKIα rescued low voluntary running behavior in a novel model of low physical activity motivation. In contrast, identical PKIα injections into the nucleus accumbens of WT rats unexpectedly did not increase their wheel running behavior and was associated with major differences in molecular responses from that seen in LVR rats, further emphasizing the existence genetic determinants specific to inactivity behavior.

Materials and Methods

Animals and Experimental Procedure

Experimental protocols were approved by the University of Missouri Animal Care and Use Committee. Rats were maintained on a 12:12-h light/dark cycle at 21–22 °C. Water and food (Formulab Diet 5008, Purina) were provided ad libitum throughout the entirety of the investigation. The authors adhered to the National Institutes of Health guide for the Care and Use of Laboratory animals (NIH Publications No. 8023, revised 1978). All efforts were made to minimize animals suffering and to reduce the number of animals used in this study. Due to their relatively high nightly running distances compared to male rats, only female LVR and WT rats were utilized [21]. Additionally, female rats were chosen based on their relatively stabilized body weight after puberty, lessening the confounding issue of increases in body weight on wheel-running behavior as in the case of male rats; as well as to build upon established work in our lab, which has primarily used female rats. Of note, running behavior in female rats is naturally entrained with their 4-day estrous cycle, in which nightly running distance peaks on the night of proestrus, a factor that was controlled for throughout this study by daily monitoring vaginal cytology and using nightly wheel-running behavior as a proxy measurement of cyclicity [22]. The founding population for the LVR line was from outbred Wistar rats (Charles River, Raleigh, NC), in which a more recent cohort was used for comparison to LVR animals. In line with the selective breeding design of Koch and Britton [23], the selective breeding paradigm for LVR rats consisted of a 13 family line. For each generation, all LVR rats were given free wheel-access (Tecniplast 2154, Tecniplast, Italy—Circumference 1.062) from 28 to 34 days of age as a necessary effort to verify low voluntary wheel-running phenotype, as previously performed by us in publications [6, 7, 17, 24–26]. To minimize potential confounding problems arising from this screening process, future investigations addressing WT and LVR rat comparisons will include an identical 6-day wheel-exposure between 28 and 34 days of age for WT rats as well. Selection was then based on nightly running distance and time, as monitored by Sigma Sport BC 800 bicycle computers, on nights 5 and 6. Similar to the breeding scheme of Garland et al., the lowest male and lowest female from each family were then chosen as breeders for the next generation [27]. The time lines of

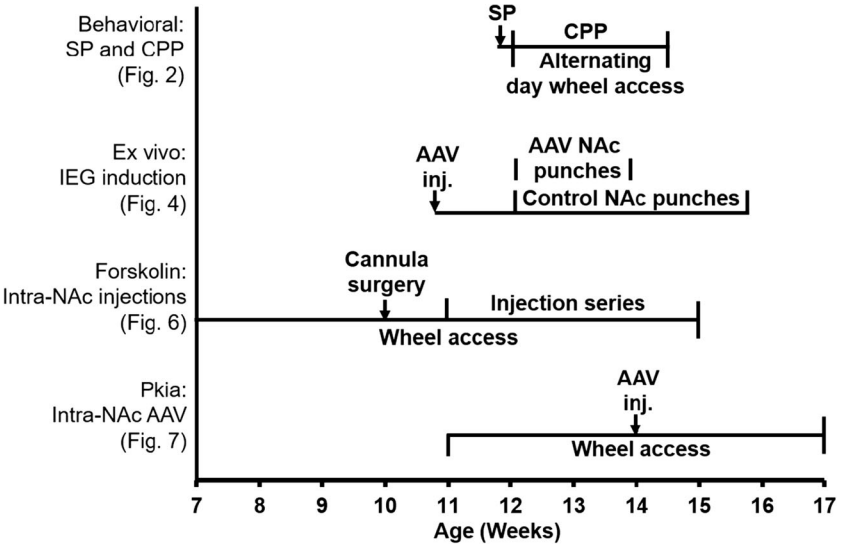

Fig. 1 Overarching experimental timelines for all animal related studies. Presented from top to bottom are the individual timelines for: Behavioral Testing-Sucrose Preference (SP) and Conditioned Place Preference (CPP); the Ex vivo—Utilizing both non-transfected control and PKIα AAV overexpressed LVR and WT NAc slices; Foskolin—The effect of intra-nucleus accumbens forskolin injection on nightly wheel-running behavior; and PKIα—The effect of NAc PKIα overexpression on wheel-running behavior. X-axis corresponds to the age of the rats (weeks) used throughout each experiment

procedures employed in four separate experiments, with each experiment using a separate set of rats are shown in Fig. 1, as Experiment #1: Behavioral: SP and CPP (Fig. 2); Experiment #2: Ex vivo: IEG induction (Fig. 4); Experiment #3 Forskolin: Intra-NAc injections (Fig. 6); and Experiment #4: Pkiα:Intra-NAc AAV (Fig. 7).

Behavioral Tests

Sucrose preference test: A subset of LVR ($n = 7$) and WT ($n = 8$) female rats (12–14 weeks of age) were used for behavioral and baseline molecular measurements, in order to confirm low expression of PKIα in LVR rats. Animals were single housed with access to voluntary running wheels on the night of proestrus, and remained single housed for the remainder of sucrose preference and conditioned place preference testing. Each rat was given a two-bottle choice of either 250 ml of tap water or 250 ml of 1% sucrose solution between 1800 and 0800, while food continued to be given ad libitum [28]. Sucrose preference was calculated as a percentage of the volume of sucrose consumed over the total volume of fluid intake over the 14-h testing period, between 1800 h (lights out) and 0800 h (2 h after lights on). Animals were returned to a standard wheel-running cage, consisting of a single bottle of tap water and standard chow given ad libitum for 7–8 weeks, during which conditioned place preference testing took place.

Conditioned place preference (CPP): The conditioning apparatus (90 cm × 30 cm × 30 cm) was made of white, treated particle board covered in 0.635-cm, clear Plexiglass and consisted of two joined compartments separated by a central probe trial starting chamber. The two compartments were separated from the central chamber by removable white Plexiglass dividers (1-cm thick). Each side of the conditioning apparatus was made to be distinctive by changing the walls and floors. The first compartment contained black horizontal strips (2.5-cm wide, 2.5-cm apart) and a floor made of clear, textured plastic flooring. The second chamber contained black vertical stripes (1.5-cm wide, 2.5-cm apart) covering both the walls and floor, over which a smooth, clear plastic flooring was placed.

The timeline of CPP training, probe trials and extinction is laid above Fig. 2c. Following 20-days of continuous wheel-exposure, conditioning training occurred daily for 16-days and consisted of alternating exposure to each of the conditioning chambers in association with either open wheel-access or wheel-lock, similar to the design of Greenwood et al. [13]. In order to determine baseline preference for either chamber of the CPP apparatus, a probe trial was performed before the start of conditioning. This trial revealed no inherent preference for either chamber; therefore, wheel-access was arbitrarily paired with the black horizontal striped chamber (paired), while wheel-lock was paired with the black vertical striped chamber (unpaired) (Fig. 2e, f). During paired conditioning, each rat ($n = 8$ WT; $n = 7$ LVR) was placed into the CPP chamber for 20 min both before lights-out (1800 h) and 20 min following overnight wheel-access (0600 h). During unpaired conditioning, wheels were locked with metal rods 1 h prior to conditioning and remained immobile overnight. In the same fashion, unpaired conditioning occurred before lights-out (1800 h) and again the following morning (0600). This time, each rat was placed in the side

of the conditioning chambers containing vertical stripes (unpaired side) for 20 min prior to lights-out and again immediately after lights-on.

Probe trials for CPP testing occurred prior to the start of the experiment to determine baseline preference, and again after 8 (trial A) and 16 days (trial B), in order to adhere to the 4-day estrous cycle. Similar to the procedure of Greenwood et al. [13], probe trials occurred between 0700 and 0800. The testing began when the dividers separating the two chambers were removed. Each rat was then given free access to both conditioning chambers for 10 min, while being continuously recorded. The amount of time spent in each chamber was monitored and recorded, in which a rat was considered to be in a chamber if all four paws were inside [13]. Each rat was tested in the same conditioning apparatus where training occurred, as well as in the same room that conditioning had occurred. CPP extinction occurred immediately following the third probe trial at 16-days (trial B), and consisted of eight continuous days of wheel-lock. A 10-min probe trial (trial C) identical to those described above occurred between 0700 and 0800. Following extinction testing, animals were sacrificed between 1700 and 1900 via CO_2 asphyxiation.

mRNA Analysis

RNA isolation and cDNA synthesis: Following sacrifice, brains were quickly removed and coronal slices of the striatum were taken using an acrylic matrix (Braintree Scientific, Braintree, MA). Two-millimeter thick and 3-mm diameter punches of the NAc, identified according to a rat brain atlas [29], were placed in 400 μL of ice-cold TRIzol reagent (Invitrogen, Carlsbad, CA) and stored at − 80 °C until processing. Upon processing, samples were lysed in Trizol reagent using RNase-free stainless steel beads and shaken three times at 25 Hz for 2 × 1-min using a high-speed homogenizer (Tissuelyser LT, Qiagen, Valencia, CA). RNA was then separated according to manufacturer's instructions (TRIzol, Invitrogen, Carlsbad, CA). RNA was quantified using Nanodrop 1000 (Thermo Scientific), and the lack of RNA degradation was verified using a 1% agarose gel. One microgram of RNA was DNase treated using DNase I (Thermo Scientific, Glen Burnie, MD) and reverse transcribed using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Carlsbad, CA).

qRT-PCR for nucleus accumbens mRNA expression: Gene-specific primers were constructed using Primer3 (Table 1), and efficiency curves were produced for all primers (acceptance being between 90 and 110% for all genes). Fifteen nanograms of cDNA from each sample were assayed in duplicate for the target genes shown in Table 1 using iTaq Universal SYBR Green Supermix (Bio-Rad, Hercules, CA). mRNA expression values were quantified using the $2^{\Delta\Delta Ct}$ method, in which ΔCt = 18S Ct – gene of interest Ct. Values were then normalized to either WT or LVR control, depending on comparison. The range of fold changes was calculated from the standard error of the ΔΔCt values.

Using Molecular Indices to Assess the Role of PKIα on Dopamine Receptor 1 (D1) Downstream Signaling

Given the role of PKIα in altering PKA signaling, and by extension D1 signaling events, we thought that it is necessary to evaluate the extent to which PKIα influences (a) AP-1 promoter activity in PC12 (pheochromocytoma) cells previously transfected with shRNA knock-down or overexpression of PKIα, following D1 stimulation with the D1-like agonist, SKF-38393 (Abcam, Cambridge, UK) and (b) immediate-early gene induction in an ex vivo preparation of the NAc of WT ($n = 8$) and LVR ($n = 7$) animals, again following D1 activation with SKF-38393. Non-running 12–14 week-old female WT and LVR animals were used in the ex vivo application. A separate cohort of WT ($n = 8$) and LVR ($n = 8$) females, which had undergone the exact stereotactic injection of an AAV for the overexpression of PKIα to be detailed in the later viral overexpression section, were used to assess the role of PKIα on immediate-early gene (IEG) expression following D1 activation in the NAc by means of the same ex vivo experimental approach.

PC12 Cell Culture and Differentiation

PC12 cells were purchased from (American Type Culture Collection, Manassas, VA). Apart from adherence to manufacturer's recommendations, further optimization of PC12 care was guided by previously established methodology [30, 31]. In general, PC12 cells were maintained on 15-mm collagen-coated culture dishes with DMEM supplemented with 10% horse serum and 5% fetal bovine serum at 37 °C in a humidified atmosphere (10% CO_2, 90% air). Medium was changed every other day, and cells were plated at an appropriate density (~ 1×10^6 cells/ml). Following plasmid-based transfection (addressed in the next section), PC12 differentiation was initiated via serum starving with 0.1% horse serum and supplementing with 50 ng/mL neural growth factor (Thermo Fisher Scientific # 13257019) until luciferase assay testing 7 days later.

AP-1 Transient Transfection and Luciferase Reporter Gene Assay

The transfection protocol follows a previously established method in our lab [32, 33]. Transient transfections of PC12 cells, including either the overexpression or the shRNA knock-down of PKIα, were performed by using the

Table 1 Primer sequences used for gene expression analysis by qRT-PCR

Gene	Forward (5′-3′)	Reverse (5′-3′)	Accession number
18S	GCCGCTAGAGGTGAAATTCTTG	CATTCTTGGCAAATGCTTTCG	NR_046237
Pkia (total)	GATATTAACAAGACAGAAGGT	GGGCTTCGCCACTTAGTT	NM_053772
Drd1	TCTCCTGGGCAATACCCTTGT	GGACCTCAGGTGTCGAAACC	NM_012546
Drd2	GTCCTGGTACGATGACGATCTG	CCTTCCCTTCTGACCCATTG	NM_012547
Fos	GGAGCCGGTCAAGAACATTA	ATGATGCCGGAAACAAGAAG	NM_022197
Pkia (endo)	CCATTGGAACAGGATCCCAGAT	ACAGTGCTCTTTACAGGGGC	NM_053772
Homer1	CTGAAGCTGGCTTTTGCGTT	TTTGAAAAGGTTGCGCGGAG	NM_031707
Zif268	CTATACTGGCCGCTTCTCCC	TTGGAGGGTTGGTCATGCTC	NM_012551
Arc	GAACCTCATCCACACCCCAG	ACCAATGGACCAGGCAGATG	NM_019361
Psd-95	GCCAATTCTCCCCCTGTGATT	CTGTTCCATTCACCTGCAACTC	NM_019621

Lipofectamine 2000 reagent (Life Technologies) along manufacturer's guidelines. Initially, transfections were carried out in 24-well tissue culture plates (77 ×104 cells/well) 24 h after seeding in antibiotic-free growth media. Vector amounts per well were as follows: 0.7 μg AP-1 reporter construct (Addgene # 40,342, Cambridge, MA) [34], 0.15 μg of Renilla pRL-null (Promega, E227A, Madison, WI), 0.3 μg of Pkia overexpression vector (VectorBuilder VB170629-1128fgt, Santa Clara, CA), or 0.3 μg Pkia shRNA in pSicoR (Addgene # 11579, shRNA target sequence GCAATGAA TTAGCCTTGAA) [35]. Six hours after transfection, the culture medium was changed to fresh PC12 growth media. The next day, cells were switched to differentiation media consisting of 0.1% horse serum plus 50 ng/mL neural growth factor in DMEM. Media was refreshed every 2-days until cells were fully differentiated 7 days later. Luciferase activities were measured with the Dual-Luciferase Reporter Assay system (Promega) according to the manufacturer's recommendations on the Veritas Microplate Luminometer (Turner BioSytems, Sunnyvale, CA).

Ex Vivo NAc Slice Preparation

To address the role of PKIα on dopaminergic downstream signaling events, two separate cohorts of LVR and WT rats were utilized to consider both endogenous responses, as well as responses following AAV PKIα overexpression. The protocol was largely adapted from Hoffman et al. [36]. Brain removal was similar to that mentioned previously, after which brains were rapidly placed in ice-cold, oxygen-saturated (O_2/CO_2: 95/5%) artificial spinal fluid (ACSF). The brains were sliced at 4 °C in a brain matrix (Braintree Scientific, Inc., Braintree, MA) into 0.5-mm-thick coronal slices. Striatal slices were transferred to an ice-cold ACSF bath where the anterior commissure was used as a reference to identify and isolate the NAc using a 2-mm tool punch (Paxinos 1998). Each slice was transferred into a 2-ml incubation tube containing 1 ml of ice-cold ACSF. The incubation tubes were gently allowed to rise to 23 °C for 30 min, after which ACSF was removed and substituted by 2 ml of fresh, oxygenated ACSF at 23 °C. Slices were left for 90 min with constant oxygenation at 34 °C. Half an hour before the addition of SKF-38393 or control ACSF, the total volume of buffer was replaced by 200 ml of oxygenated ACSF (34 °C). SKF-38393 was dissolved at 2× the final concentration. Two hundred microliters of SKF-38393 solution or fresh ACSF was gently added to the incubation tube for 10 min. Incubation was ended by removing the complete volume of liquid and flash freezing the slices in liquid nitrogen. mRNA processing of slices occurred according to aforementioned mRNA method section.

Pharmacological Manipulation of Nucleus Accumbens PKA Signaling

In order to evaluate the role of nucleus accumbens PKA signaling on wheel-running behavior, a separate set of female WT ($n = 8$) and LVR ($n = 7$) rats were used to determine if intra-nucleus accumbens injections of adenylyl cyclase activator Forskolin (0.25 or 1.0 μg/0.5 μl) influenced nightly wheel-running distance on the night of proestrus. Rats were weaned at 21 days of age and given free access to voluntary running wheels at 9 weeks of age for 3 weeks. Running behavior of each rat was monitored over these 3 weeks to determine nightly running periodicity, a characteristic that is entrained to their estrus cycle [22]. As such, intra-nucleus accumbens injections were performed on the night of proestrus in a repeated measures design [6, 24].

On the day of the surgery, rats (250–300 g) were anesthetized with 2% isoflurane. After shaving off their heads, animals were positioned in a stereotaxic frame (David Kopf Instruments, Tunjunga, CA) and 10-mm, 23-gauge guide cannulae were bilaterally positioned 2.5 mm above the NAc core via the following coordinates: relative to Bregma-anteroposterior (AP) 1.4 mm, mediolateral (ML) ± 1.85 mm, and dorsoventral (DV) − 5.0 mm [37]. Skull screws and dental cement were used to secure guide cannulae, after proper

drying of dental cement, 10-mm, 30-gauge stylets were inserted into the guide cannulae to prevent obstructions. Following surgery, topical Neosporin was applied around the surgical region and animals were warmed on a 32 °C heating pad until ambulatory. After initial recovery, rats were returned to their home cages with running-wheels and monitored for the following week to confirm that running patterns returned to pre-surgical levels.

In line with a previously used methods in our lab, drug injections of forskolin occurred 30 min prior to the start of the dark cycle of the night of proestrus, previously established to be the highest night of wheel-running distance [6, 24]. During injections, rats were gently hand-restrained for 120 s and 10-mm Hamilton syringes were mounted to an infusion pump (Harvard Apparatus, Holliston, MA). 12.5-mm, 30-gauge injector cannulae were connected to the Hamilton syringes with PE-10 tubing that was used to deliver either (a) sterile vehicle (VEH) or (b) forskolin (Tocris, Bristol, UK) at a rate of 0.25 μl/min in a counter-balanced fashion. The injectors were left in place for 60 s following the end of injection to ensure that saline/drug was appropriately infused. Following completion of the injections, rats were returned to their home cages to monitor nightly wheel running behavior. Drug concentrations were determined from a pilot study using LVR ($n = 3$) and WT rats ($n = 3$), which suggested the doses used could affect wheel-running (unpublished).

Following a previously established method [38], each rat functioned as its own control in a repeated measure design in which each rat was serially injected with either VEH or forskolin in a randomized order on the night of proestrus for three continuous estrus cycles. Following the guidelines of Ruegsegger et al. [6] to ensure no handling or persistent drug effects were present, the following precautions were taken: (a) VEH injections were performed prior to each drug schedule in order to limit the effect of handling or drug on running distances, (b) running distances during the night following each injection were compared across injection schedule to determine if treatment effected nightly running distance, and (c) VEH was injected at the end of each drug schedule and compared to previous VEH nights to verify animals did not change their running during the course of the study.

Cannulae placement was determined according to the methods of Will et al. [39], Following the final saline injection, rats were sacrificed via CO_2 asphyxiation and transcardially perfused with 4% paraformaldehyde in 0.1-M phosphate buffer. After removal, brains were stored in 4% paraformaldehyde overnight at 4 °C. For cryoprotection, brains were placed in 30% sucrose in 0.1 M phosphate buffer at 4 °C until resting in solution (48–72 h), after which were frozen until processing. For sectioning, brains were blocked and mounted in OCT media, left frozen for 20 min in a cryostat, and sectioned in the coronal plane, yielding 40-μm-thick slices. After visualizing injector tracks, slices were mounted on charged microscopy slides, stained with 1% cresyl violet, and examined using a light microscope to confirm correct cannulae placement. The placement of each injector was mapped on a rat brain atlas [29], as shown in supplemental Fig. 1.

Viral Overexpression of Nucleus Accumbens PKIα

To assess the role of NAc PKIα expression on voluntary wheel-running behavior, a separate set of 11-week-old female WT ($n = 10$) and LVR ($n = 8$) animals previously given 3 weeks of voluntary wheel-access were injected with either an adeno-associated virus (AAV) expressing an empty-vector or an AAV for the overexpression of PKIα within the NAc core. AAV particles were designed and created by VectorBuilder-Cyagen Biosciences Inc. PKIα AAV1 expression is driven by a chicken beta actin promoter with GFP independently driven by IRES. WT and LVR animals underwent stereotactic surgery (David Kopf Instruments, Tujunga, CA) under 2% isoflurane anesthetic for the NAc-specific injection of AAV expressing PKIα or an AAV expressing an empty vector to function as a positive control (relative to Bregma; A, 1.4 mm; L, 1.5 mm lateral to the midline suture; − 7.5 mm below the skull [40]. Viral injection consisted of 1 μL of virus (~ 10^{12} GC/ml) infused unilaterally (stereotaxic injector-Stoelting Co) (total of 2 μL per animal) over the course of 3 min with an additional 2 min to allow for proper diffusion. Similar AAV infusion rates have been successfully used in our lab [41]. Following injections, incisions were closed using tissue adhesive (Vetbond, 3 M, Maplewood, MN), and rats were allowed to recover on a 32 °C heating pad until ambulatory. After initial recovery, rats were returned to their home cages with running-wheels and monitored for the following week to confirm running patterns returned to pre-surgical levels. After 1 week of recovery, running distance was recorded for 20 days (~ 5 estrous cycles). Overexpression of PKIα was verified via qRT-PCR according to the methods laid out previously.

Statistical Analysis

Analyses were performed using SigmaPlot 12.0 (Systat Software, Inc., Chicago, IL). All values are presented as mean ± SE. Significance for all analyses were set to an alpha value of 0.05. Within group variables (effects of repeated NAc-specific injections on nightly running distance) were analyzed using one-way repeated measures ANOVAs. Significant main effects (effect of PKIα overexpression or SKF-38393 treatment) were further analyzed by Holm-Sidak post hoc comparisons. Student's *t* test was used to compare between-line (LVR vs WT) differences in running distance.

Results

Reward-related behavioral testing: LVR rats show similar sucrose preference and conditioned place preference for long-term wheel-running compared to WT rats, despite LVR rats having a lower volume of running and sucrose consumption.

In order to address the potential that LVR rats may have decreased reward-incentive motivation to voluntarily wheel-run because of a general lack of ability to experience reward, or due to a lack of finding wheel-running rewarding, we first describe the behavioral experiments, and then present their results. The experimental timeline is laid out at the top of Fig. 2c for sucrose preference, used to measure baseline anhedonia [42, 43], and also for conditioned place preference in Fig. 2c, which was used to test the reward-incentive properties of long-term wheel-running for both WT and LVR rats [44, 45]. Wheel-running behavior over the first 14 days of open-wheel access, as well as during the CPP testing period, is shown in Fig. 2c. Average nightly running distance was ~3-fold greater in WT compared to LVR rats in the 2 weeks prior to and during CPP (Fig. 2d), demonstrating a noticeable divergence in wheel-running motivation. Figure 2a shows no differences in sucrose preference (SP) between WT and LVR rats, implying LVR rats do not have major deficits in this reward valuation. Interestingly, LVR rats on average consumed roughly half of the volume of 1% sucrose water compared to their WT counterparts (57.056 ± 6.86 vs 111.871 ± 8.09; $p < 0.001$) (Fig. 2b) despite drinking the same percentage of sucrose solution per total volume of liquid (Fig. 2a). This lower overall sucrose consumption suggesting a potential decrease in consummatory behavior for this natural reward, which may support the similarly low running distance of LVR rats.

During baseline (BL on x-axis) CPP probe trial testing, both WT and LVR rats spent equal amounts of time in both sides of the CPP apparatus, signifying no inherent bias of chamber for either group prior to conditioning (Fig. 2e, f). There was no difference in the time spent in either chamber for WT rats at the first probe trial time point (WT trial A-Fig. 2e). However, in contradiction, LVR rats spent significantly more time in the paired versus in the unpaired side at this point (LVR trial A-Fig. 2f). During the probe trial at 16-days past the beginning of conditioning (trail B), both WT and LVR animals spent significantly more time in the chamber paired to open wheel-access compared to the unpaired side, signifying an increased preference for open-wheel access compared to wheel-lock in both groups (Fig. 2e, f). The final probe trial following extinction (trial C), consisting of 8 days of

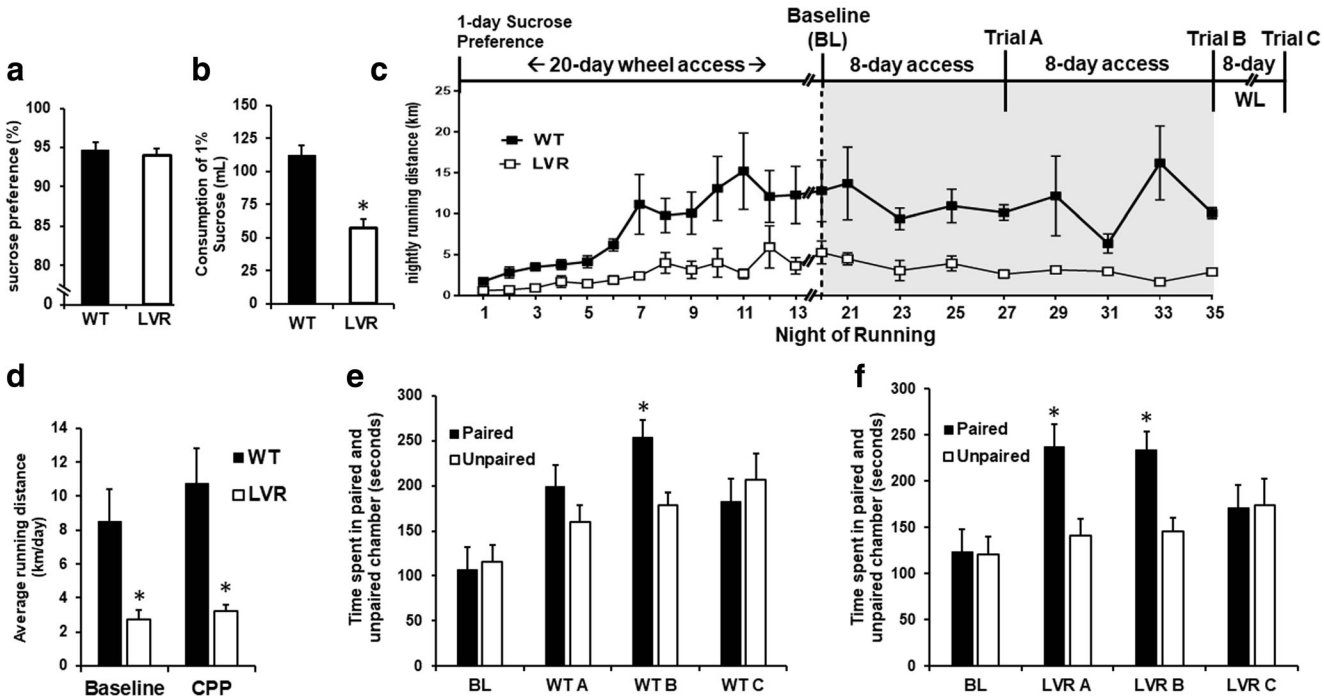

Fig. 2 (At top of **c**) Experimental timeline for 1% sucrose preference (**a**–**b**) and conditioned place preference (CPP) (**c**–**f**). Following 20 days of wheel-running a baseline (BL) probe test was taken. During CPP training, wheel-running was paired (20 min before and after the dark cycle) with one side of the chamber, and wheel-lock (WL) with the unpaired side. Ten-minute probe trials occurred after 8 days of training (trial A) and 16 days (trial B), which was followed by 8 days of wheel-lock extinction (trial C) in low voluntary runners (LVR) and wild-type (WT) groups. **d** Average nightly running distance (km) for WT and LVR rats over 14-day wheel-access (baseline), followed by 16 days of alternating open-wheel-access and wheel-lock (CPP) (± SEM). **e** For WT ($n = 8$) rats, mean time (seconds) they spent in paired and unpaired sides in BL and probe trails A–C. **f** For LVR ($n = 7$) rats, mean time (seconds) they spent in paired and unpaired sides in BL and probe trails A–C. Symbols, * denotes a significant difference between WT and LVR values or time spent in paired compared to unpaired chamber ($p < 0.05$)

continuous wheel-lock, showed no difference in time spent in either the paired or the unpaired chamber for either WT (Fig. 2e) or LVR (Fig. 2f) rats, suggesting the learned association between open wheel-access and the paired chamber had been lost for both groups.

Verification of PKIα Expression Differences Between LVR and WT Rats and Its Correlation with Nightly Wheel-Running Distance

Building upon our previously published RNA-sequencing study addressing divergences in NAc transcripts between LVR and our high-voluntary running (HVR) model at 4 weeks of age [5], we utilized the same LVR and WT rats that underwent CPP conditioning (Fig. 2) to verify the difference in expression of PKIα between 12 and 14-week-old LVR and WT rats, an age which is more representative of a reproductive population [46]. As expected, LVR NAc expression of PKIα was 20% lower compared to WT rats ($p = 0.013$) (Fig. 3a), similar to our previous findings of this difference (unpublished finding). To further appreciate the relationship between PKIα expression and nightly running behavior, correlation to nightly run distance on the night of proestrus prior to the start of CPP training was assessed. When considering LVR and WT rats collectively, there was an overall positive correlation between Pkiα mRNA expression and nightly run distance on the night of proestrus ($r = 0.66$; $p < 0.01$; Fig. 3b), but not on the other nights of the estrus cycle. There was no correlation between nightly distance and NAc PKIα expression for either WT ($r = 0.42$; $p = 0.30$) or LVR ($r = 0.47$; $p = 0.29$) rats alone (Fig. 3b). The preliminary results supported later experiments for gene therapy with PKIα.

In order to verify that endogenous differences in PKIα expression existed between WT and LVR rats independent of the influence of wheel-running, control values of PKIα expression were determined in ex vivo-treated NAc slices taken from sedentary animals (Fig. 4a). Consistent with expression differences between the CPP LVR and WT cohorts (Fig. 3a), LVR showed ~ 60% lower expression of PKIα compared to sedentary WT rats (0.634 ± 0.092 vs 1.0 ± 0.125; $p = 0.05$) (Fig. 4a). The aforementioned importantly suggests that the divergence in PKIα is an existing endogenous difference, and not simply an acute response to different amounts of wheel-running between LVR and WT rats. One small caveat being that only LVR had a brief 6-day wheel running exposure in their 5th week of life to verify phenotype, and remained sedentary until sacrifice.

Non-Transfected LVR NAc Punches Show Increased Immediate-Early Gene (IEG) Induction Following D1 Agonism, Compared to WT NAc Punches Which Shows Decreased IEG Response to D1 Agonism

To interpret the potential role of PKIα in modulating dopamine D1 signaling, and by extension PKA signaling activity, D1 induced immediate-early gene (IEG) expression in an ex vivo preparation, which functions as a molecular snapshot of D1 activity and thus motivation-related signaling in the NAc (Fig. 4b–i), was considered.

Immediate-early gene (IEG) induction in isolated NAc slices from WT and LVR animals was analyzed at both baseline and following application of D1-like agonist SKF-38393 (20 μM), to mimic the effect of increases in dopamine signaling during nightly wheel-running. The effect of D1 stimulation on IEG expression was addressed for both non-transfected (NT) WT and LVR control rats (Fig. 4b–e), as well as for PKIα virally overexpressed (OV) WT and LVR NAc slices (Fig. 4f–i). The immediate-early genes: *Homer1* (Fig. 4b, f); *Zif268* (Fig. 4c, g); *Arc* (Fig. 4d, h) and *Psd-95* (Fig. 4e, i) were considered as markers of downstream PKA activity and were chosen due to their potential involvement in motivation-related cellular changes [47, 48].

Interestingly, for non-transfected (NT) control (Ctl) NAc slices a significant line effect existed between LVR and WT

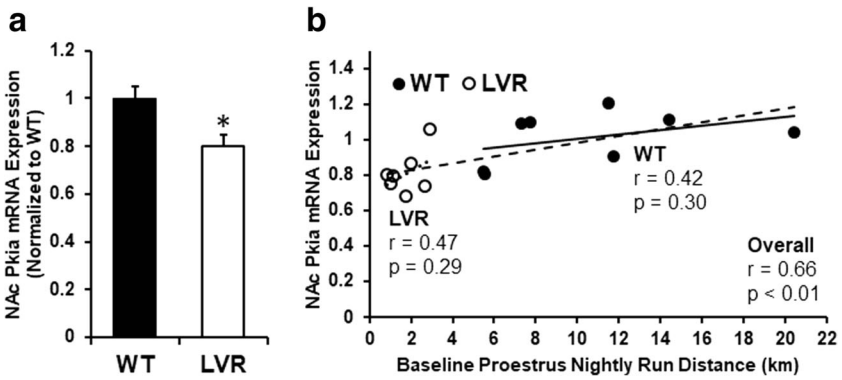

Fig. 3 **a** Relative nucleus accumbens Pkia mRNA expression, presented as $2^{\Delta\Delta CT}$, wherein ΔCT = 18S – Pkia CT, normalized to 1.0 for WT. **b** Relationship between nightly wheel-running distance on night of proestrus and Pkia mRNA expression for low voluntary runners (LVR) and wild-type (WT) groups of rats, separately and combined. * denotes statistically significant difference between groups

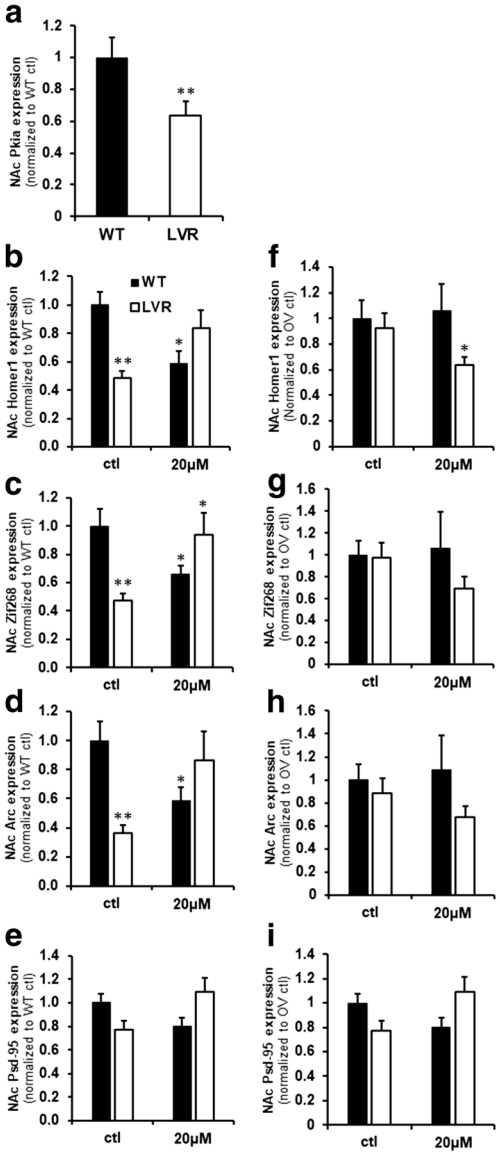

Fig. 4 Effect of PKIα expression on downstream PKA signaling indices following D1-like agonist SKF-38393 treatment. **a** Relative mRNA expression of the nucleus accumbens (NAc) transcript Pkia in control (Ctl) slices (that did not undergo treatment with SKF-38393) taken from WT and LVR rats. **b–i** Relative mRNA expression of the NAc transcripts in low voluntary runners (LVR) and wild-type (WT) groups of rats: **b**, **f** Homer1; **c**, **g** Zif268; **d**, **h** Arc; and **e**, **i** Psd-95 in non-transfected (**b–i**), as well as in transfected, PKIα overexpressed (**f–i**) WT and LVR NAc slices following 10-min SKF-38393 (20 μM) application. * denotes significantly different ($p < 0.05$) within group (ctl vs SKF-3893), ** denotes significantly different between groups (WT vs LVR)

slices, showing a lower baseline expression in LVR slices for *Homer1* (0.487 ± 0.048 vs 1.00 ± 0.094, $p < 0.001$, Fig. 4b); *Zif268* (0.472 ± 0.054 vs 1.00 ± 0.118; $p = 0.003$; Fig. 4c); and *Arc* (0.360 ± 0.06 vs 1.00 ± 0.131; $p < 0.001$; Fig. 4d) compared to WT slices; but not *Psd-95* (0.77 ± 0.078 vs 1.00 ± 0.077; $p = 0.068$; Fig. 4e). Considering these slices were from sedentary rats, the lower baseline IEG expressions could infer differences in reward valuation between LVR and WT when not given positive incentive stimuli, i.e., running wheels, though earlier baseline sucrose-preference data suggested that LVR rats are, at the very least, not anhedonic.

For WT, non-transfected (NT) NAc slices, analysis revealed a significant effect of the dopamine D1-like agonist SKF-38393 treatment on the expression of *Homer1* ($F(1,10) = 12.89$; $p = 0.005$); *Zif268* ($F(1,10) = 7.37$; $p = 0.021$); *Arc* ($F(1,10) = 6.89$; $p = 0.022$); but not *Psd-95* ($F(1,10) = 3.29$; $p = 0.094$). Post-hoc analysis revealed a lower expression of *Homer1* (1.0 ± 0.05 vs 0.586 ± 0.088; $p = 0.005$), *Zif268* (1.00 ± 0.12 vs 0.66 ± 0.054; $p = 0.026$) and *Arc* (1.00 ± 0.13 vs 0.586 ± 0.089; $p = 0.022$) in WT slices following 20 μM of SKF-38393 compared to control slices (Fig. 4b–d). For non-transfected (NT) LVR NAc slices there was a main effect of SKF-38393 application for *Homer1* ($F(1,11) = 7.67$; $p = 0.017$); *Zif268* ($F(1,11) = 7.27$; $p = 0.021$); *Arc* ($F(1,11) = 7.00$; $p = 0.023$); but not or Psd-95 ($F(1,11) = 3.174$; $p = 0.0689$). Post-hoc analysis revealed NT LVR slices following 20 μM SKF-38393 had a higher fold expression of *Homer1* (0.838 ± 0.12 vs 0.49 ± 0.048); *Zif268* (0.935 ± 0.16 vs 0.472 ± 0.05; $p = 0.02$) and *Arc* (0.866 ± 0.19 vs 0.360 ± 0.057) compared to their respective LVR control slices (Fig. 4b–d), suggesting the decreased inhibitory role of PKIα in these animals confers an increased sensitivity to D1 activation.

PKIα Overexpression Appears to Normalize LVR and WT Immediate-Early Gene Response to Dopamine D1 Receptor Agonism

To better evaluate the role of PKIα on dopaminergic downstream signaling, we considered the effect of the D1-like agonist SKF-38393 treatment on NAc ex vivo punches from LVR and WT rats following PKIα overexpression. Given the position of PKIα to influence PKA, and by extension D1 signaling, we decided to focus on D1 signaling specifically. A limitation to the study is the fact that PKIα overexpression should also increase PKIα expression in D2 neurons and D1-D2 co-expressing medium spiny neurons as well. In so, any changes in IEG expression could be due to other neuron types and not simply D1 expressing neurons. Within the above limitations, we effectively sought to assess if increasing NAc PKIα expression in LVR rats allowed similar responses to SKF-38393 as non-transfected WT slices. Intriguingly, the apparent endogenous difference in baseline IEG expression appears to be lost following PKIα overexpression (OV), in which there was no line effect between controls for *Homer1* ($F(1,11) = 0.134$; $p = 0.72$; Fig. 4f); *Zif268* ($F(1,10) = 0.013$; $p = 0.91$ Fig. 4g); *Arc* ($F(1,11) = 0.33$; $p = 0.58$; Fig. 4h), or *Psd-95* ($F(1,11) = 0.16$; $p = 0.70$; Fig. 4i), implying a potential role of PKIα in modifying motivational signaling at the IEG level. There was no significant effect of SKF-3893 treatment

for OV WT NAc slices for the expression of *Homer1* ($F(1,14) = 0.069$; $p = 0.797$); *Zif268* ($F(1,14) = 0.030$; $p = 0.865$); *Arc* ($F(1,14) = 0.076$; $p = 0.787$); or *Psd-95* ($F(1,14) = 0.002$; $p = 0.967$). Similarly, there was no effect of SKF-38393 treatment on OV LVR slices for the expression of *Zif268* ($F(1,11) = 2.439$; $p = 0.147$); *Arc* ($F(1,11) = 1.607$; $p = 0.231$); or *Psd-95* ($F(1,11) = 4.537$; $p = 0.0566$). There was, however, an effect of SKF-38393 treatment on *Homer1* expression ($F(1,11) = 5.082$; $p = 0.0455$). Post-hoc analysis a lower expression of *Homer1* in OV LVR NAc slices following 20 μM of SKF-38393 compared to non-stimulated control slices (0.638 ± 0.061 vs 0.926 ± 0.113; $p = 0.0455$; Fig. 4f).

PKIα Knock-Down and Overexpression Show a Corresponding Increase and Decrease in AP-1 Promoter Activity in PC-12 Cells

By modifying PKIα expression in a dopaminergic cell line, in part to mimic its endogenously low expression in the nucleus accumbens of LVR rats, we were able to more directly address the role of PKIα in modulating PKA activity, which is known to be a key regulator in motivation-related signaling [49]. Utilized as a molecular proof-of-principle, AP-1-driven luciferase activity in (a) control PC12 cells, (b) those transfected for the shRNA knock-down, or (c) overexpression of PKIα is shown in Fig. 5. Analysis revealed an overall effect of knockdown on luciferase activity ($F(1,16) = 40.84$; $p < 0.001$), having a greater activity level compared to control PC12 cells (1.67 ± 0.088 vs. 1.04 ± 0.045; Fig. 5), suggesting decreased expression of PKIα confer increases in PKA activity. In contrast to knockdown, overexpression of PKIα ($F(1,16) = 74.88$; $p < 0.001$), showed a noticeably lower level of AP-1 activity compared to control values (0.59 ± 0.027 vs. 1.04 ± 0.045; Fig. 5), suggesting increased PKIα expression decreases PKA activity.

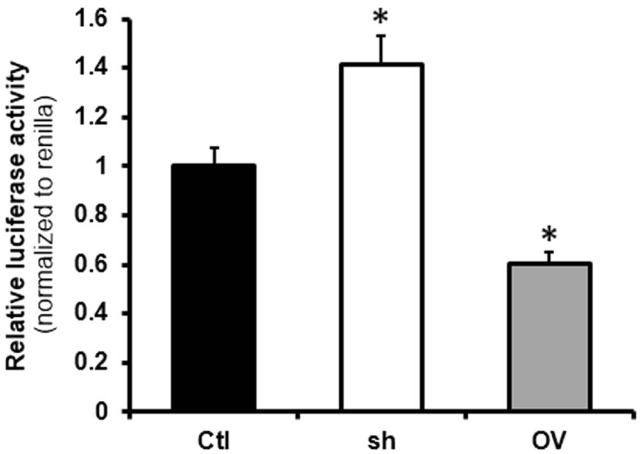

Fig. 5 Absolute AP-1 promoter activity in PC-12 cells that were previously transfected with shRNA knock-down (sh) or overexpression (OV) of PKIα. * denotes statistically significant from control (Ctl)

PKA Activation Via Forskolin Decreases Nightly Wheel-Running Behavior in WT, but Not LVR Rats

To address the role of PKA modulation on nightly wheel-running behavior, we observed the response of female WT and LVR rats to intra-nucleus accumbens infusions of adenylyl cyclase activator forskolin in a longitudinal, repeated injections study. Nightly running distances following bilateral NAc injection of either control vehicle (VEH), 250 ng of forskolin/0.5 μl, or 1 μg of forskolin/0.5 μl immediately prior to the dark cycle on the night of proestrus are shown in Fig. 6. Repeated measures ANOVA revealed a significant effect of forskolin (FRK) on nightly run distance in WT ($F(2,7) = 8.16$; $p = 0.004$), but not LVR rats. Post-hoc analysis revealed that wheel-running distance in WT was decreased compared to VEH (14.09 ± 2.89) at both the 250 ng/μl (5.75 ± 1.71, $p = 0.027$) and 1 μg/μl (6.31 ± 1.52; $p = 0.032$) doses (Fig. 6). Interestingly, there was a nearly significant higher run distance in WT following the 1-μg dose of forskolin compared to LVR at the same dose (6.312 ± 1.52 vs 2.739 ± 0.80; $p = 0.057$). Fundamentally, these findings reflect a similar masking effect seen following dopamine D1 receptor activation, largely functioning through PKA signaling, in which rats selectively bred to run high distance (HVR) decreased nightly running distances, while LVR rats did not [16]. No repeated injection effect on nightly running distance, determined by comparing the experimental control VEH injection running distance with a final VEH injection following the termination of all drug injections, was seen in either WT or LVR cohorts ($p > 0.05$, data not shown).

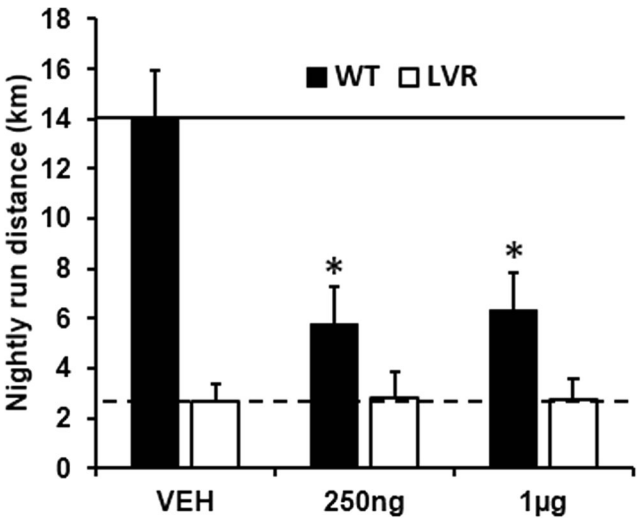

Fig. 6 Total proestrus nightly running distance following injections of forskolin, administered 30 min prior to the start of the dark cycle for WT ($n = 8$) and LVR ($n = 8$) rats. Data is presented as mean ± SEM. Symbols: * denotes effect of drug < VEH injection ($p < 0.05$) within WT and LVR lines

NAc PKIα Overexpression Increases Nightly Wheel-Running Behavior in LVR, but Not WT Rats

To better, and more directly, test the extent to which PKIα affects the motivation to voluntarily wheel-run, we employed its viral overexpression. Prior to either PKIα AAV overexpression (OV) or empty vector (EV) administration, WT and LVR cohorts were given access to voluntary running wheels for 3-weeks and pair-matched based on nightly running distance. There was no difference in average nightly running distance between WT groups chosen for OV or EV AAV NAc injections (19.78 ± 2.17 vs 18.66 ± 1.86; $p = 0.70$), nor between LVR groups chosen for OV or EV AAV surgery (1.81 ± 0.46 vs 1.81 ± 0.47; $p = 0.99$). In using the consistently highest 4th night of running behavior as a proxy for proestrus in rats, running patterns were lined according to each animal's corresponding estrus cycle. The two estrus cycles prior to AAV injection surgery and the 20-days (five estrus cycles) following the week of recovery following surgery for WT and LVR rats are shown in Fig. 7a, b, respectively. Post-recovery nightly running behavior is shown to the right of the vertical dashed line for both WT (Fig. 7a) and LVR (Fig. 7b) rats. There was no main effect of PKIα overexpression on average nightly running distance for WT ($F(1,18) = 1.50$; $p = 0.24$) or LVR ($F(1,15) = 4.01$; $p = 0.064$) rats over the course of the 20-day, five-estrus cycle observation. However, there was an effect of PKIα overexpression on nightly running distance on the night of proestrus for LVR ($F(1,15) = 5.14$; $p = 0.039$), but not WT rats ($F(1,18) = 2.40$; $p = 0.14$). In considering average nightly running behavior per 4-day estrus cycle, post-hoc analysis revealed OV LVR rats ran more than their EV counterparts on estrus cycles 1 (4.52 ± 1.3 km vs 1.61 ± 0.35 km; $p = 0.038$), 4 (9.46 ± 1.96 vs 2.75 ± 0.58; $p = 0.004$), and 5 (9.78 ± 1.97 vs 2.86 ± 0.57; $p = 005$; Fig. 7b). Considering the total amount of distance ran post AAV surgery, LVR OV rats ran 3.1-fold higher than their EV counterparts over the course of the 20-day running period (3.13 ± 0.90 vs 1.00 ± 0.220; $p = 0.031$; Fig. 7c).

To validate the effectiveness of the AAV and confirm proper overexpression, mRNA expression of *Pkia* was assessed via qRT-PCR (Fig. 7d). There was only a near significant fold increase in PKIα mRNA expression in WT OV compared to

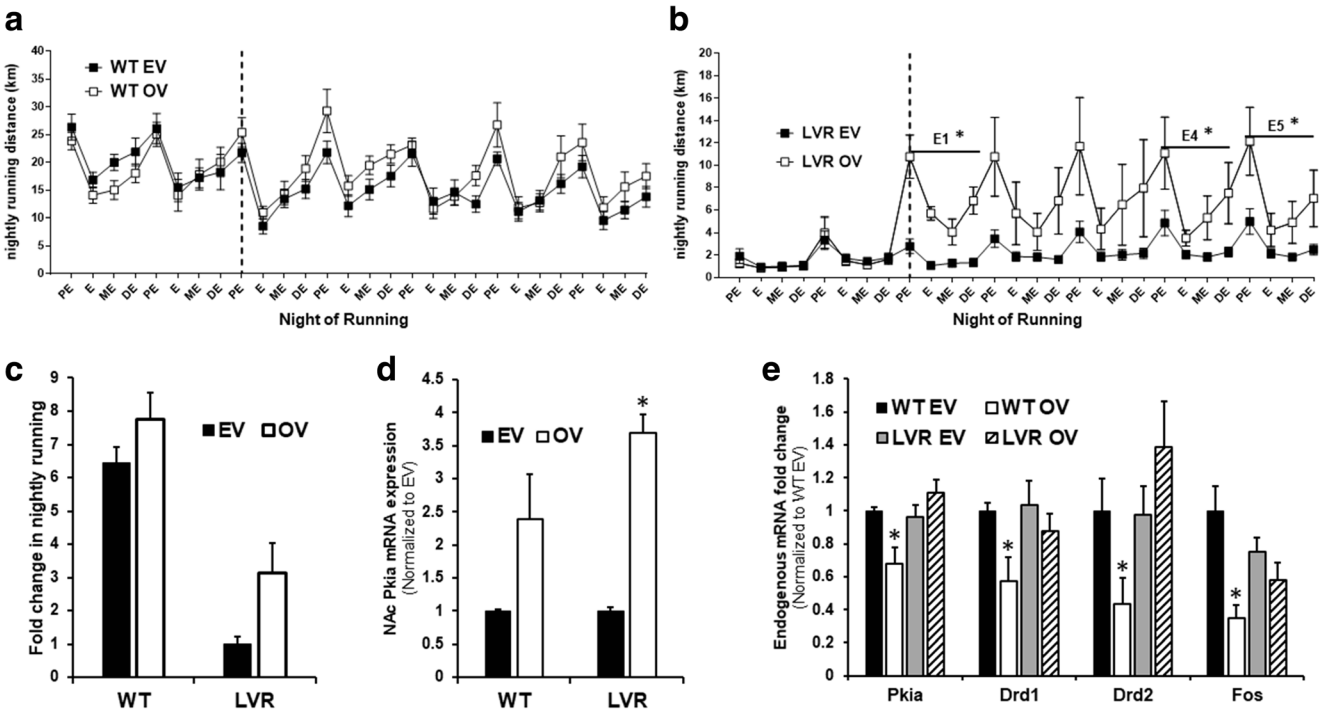

Fig. 7 Effect of nucleus accumbens (NAc) PKIα overexpression on nightly wheel-running behavior and dopamine-related transcript expression in low voluntary runners (LVR) and wild-type (WT) rats. **a** Nightly running distance (km) in female WT rats during baseline (left of vertical, broken line) or following NAc injection of adenoviral-associated (AAV) expressing either empty-vector (EV-closed; $n = 10$) or PKIα gene overexpression (OV-open; $n = 10$). **b** Nightly running distance (km) in female LVR rats during baseline (left of vertical, broken line) or following NAc injection of AAV expressing either empty-vector (EV-closed; $n = 9$) or PKIα overexpression (OV-open; $n = 8$). **c** Total fold-change in nightly run distance over the 20-day post-injection running period for WT and LVR rats following EV and OV injections, normalized to LVR EV run distance. **d** Verification of total PKIα overexpression. Relative mRNA expression of Pkia for EV and OV NAc punches in WT and LVR rats, normalized to respective EV groups. **e** Relative endogenous mRNA expression of Pkia (determined by UTR region, which was not expressed in AAV overexpression vector); Drd1; Drd2; and Fos. * denotes statistically different ($p < 0.05$) between treatment groups (EV vs OV)

EV animals (2.38 ± 0.031 vs 1.0 ± 0.68; $p = 0.07$). However, LVR OV NAc punches did show a greater expression of PKIα mRNA compared to LVR EV punches (4.03 ± 0.30 vs 1.09 ± 0.052; $p < 0.001$).

Given the stark differences in wheel-running responses to PKIα overexpression between LVR and WT rats, the effect of its overexpression on molecular markers associated with dopamine signaling were also addressed to help explain the lack of wheel-running distance response in WT rats. Due to their involvement in establishing motivation and reward, we examined the mRNA expressions of dopamine D1 receptor (Drd1), dopamine D2 receptor (Drd2) and Fos (Fig. 7e). There was a main effect of PKIα overexpression on mRNAs of Drd1 ($F(1,33) = 6.4$; $p = 0.016$) and Fos ($F(1,33) = 16.4$; $p < 0.001$) expression, but not Drd2 expression ($F(1,33) = 0.50$; $p = 0.486$). Post-hoc analysis revealed a significantly lower NAc mRNA expressions of Drd1 in WT OV compared to WT EV groups (1.0 ± 0.05 vs 0.57 ± 0.15; $p = 0.015$; Fig. 7e), as well as for Drd2 (1.0 ± 0.19 vs 0.43 ± 0.16; $p < 0.01$; Fig. 5e) and Fos (1.0 ± 0.15 vs 0.35 ± 0.08; $p < 0.01$; Fig. 7e). However, there was no difference in expression between LVR OV and LVR EV NAc expression for any of the aforementioned genes. The effect of PKIα overexpression on endogenous PKIα mRNA expression within the NAc, as measured by mRNA expression of the untranslated region (UTR), was also considered (Fig. 7e). There was no main effect of PKIα overexpression on endogenous PKIα mRNA expression. Interestingly, there was a lower endogenous PKIα mRNA expression in WT OV compared to WT EV rats ($F(1,17) = 9.18$; $p = 0.008$) following PKIα overexpression, which may help support the lack of verified PKIα overexpression in WT punches (Fig. 7d).

Discussion

As physical inactivity is highly prevalent [2] and responsible for at least 20% of prominent chronic diseases (http://www.who.int/dietphysicalactivity/pa/en/), a therapy to reduce inactivity would greatly improve our health and the quality of life [50]. The present study culminated in an experiment that identified protein kinase inhibitor alpha (PKIA) as a gene candidate, and when overexpressed in the NAc partially rescued low voluntary running behavior distance in rats selectively bred for the phenotype of low voluntary running behavior (LVR). A finding that was longitudinally repeated and measured over five-estrus cycles. Surprisingly, WT rats showed a behavioral and molecular resistance for localized overexpression of PKIα. Taken together, the above, unique findings suggest the likely existence of a gene with a function in the NAc specific to the modulation of low physical activity motivation.

Identification of PKIα as a Candidate Gene for Low Voluntary Running Distance

The roughly 3-fold divergence in nightly wheel-running distance between LVR and WT rats (Fig. 3b) is consistent within the previous magnitudes for larger comparisons made between LVR and high voluntary running (HVR) rats in our lab [5, 6]. Herein, the PKA modulator, PKIα, was ~ 20% more highly expressed in NAc of WT compared to LVR rats (Fig. 3a). While correlation does not prove causation, Pkiα mRNA level was positively associated with wheel-running distance on the night of proestrus, supporting a potential role for this molecule in wheel-running motivation (Fig. 3b). In total, our above preliminary evidence was judged to be sufficient for PKIα to be designated as a candidate gene for further experimentation.

LVR Rats Find Wheel-Running Rewarding

Working from the framework that rats are motivated to voluntarily wheel-run because of an underlying positive or rewarding value [13, 51], we felt it necessary to determine if the decreased wheel-running motivation seen in LVR rats stems from a lower level of reward valuation. From this perspective, one goal of the next experiment was to determine if LVR rats express the same incentive salience for wheel-running reward as in non-selected, wild-type (WT) rats. Additionally, we wanted to address the possibility that our LVR rats are anhedonic, i.e., unable to experience reward, which would explain the exceptionally low level of voluntary wheel-running behavior [52]. To test the possibility, WT and LVR rats were given a sucrose preference test, a measure of anhedonia in rodents [42, 43], prior to the acquisition of voluntary running wheels. However, no difference was seen between WT and LVR rats for sucrose preference, implying our LVR rats do experience reward.

Further assessment of 1% sucrose intake revealed that, on average, WT rats consumed more sucrose solution compared to LVR rats, despite the percent preference for sucrose being the same. From the perspective of consummatory behavior, this finding suggests that both total running distance, which could be viewed as "consumption" of wheel-running, and total sucrose intake are inherently lower in LVR compared to WT rats. The nuances of sucrose preference and intake are controversial in relationship to human reward-valuation [52]. In so, further work is needed to better understand and address whether the consummatory behavior of LVR rats is generalized to natural reward, or specifically voluntary wheel-running behavior.

Conditioned place preference (CPP) is a test more specific to the rewarding value of long-term wheel running in LVR rats, while also serving to compliment the goal of sucrose preference testing [13, 44, 53]. Unexpectedly, LVR rats

appeared to show an increased preference for voluntary-running wheel access at an earlier time point compared to their WT counterparts. Upon the second trial of CPP, both WT and LVR rats showed roughly the same preference for wheel-access. Considering that motivation is goal-oriented, in this case the goal being the reward from wheel-running, the above suggests that LVR rats do find wheel-running rewarding, but by inference are likely less motivated to do so. Taken together, the finding of similar wheel-running rewards led us to the next experiments to address potential molecular underpinnings that could contribute to the lack of motivation to voluntarily wheel-run in LVR rats.

NAc Downstream Signaling Differences Between LVR and WT, and the Effect of PKIα Expression on IEG Induction

Association between upstream D1 signaling and PKIα expression levels in control WT and LVR rats, as well as following PKIα overexpression in the NAc were performed. In order to index D1 activity as a marker of potential dopamine signaling, and by extension PKA activity, the immediate-early genes (IEG) *Homer1*, *Zif268*, *Arc*, and *Psd-95* were determined. Attention to these genes stemmed from their previously reported involvement in changes in neuroplasticity that underlie reward and addiction [54]. The underlying rationale was that IEG determinations could enlighten our experimental efforts in understanding dopamine-mediated long-term plasticity, cellular changes associated with glutamatergic receptor expression, and downstream-mediated synaptic excitability, all of which have implications in reward and motivation [47, 48]. A limitation to the focus of the study being on D1 signaling exclusively is that PKIα overexpression likely increased in D2 as well as D1-D2 co-expressing neurons in the NAc. As such, future investigations are needed to better address the role of PKIα in influencing downstream signaling events in the various NAc neuronal populations as a whole, as well as the significance of these in relationship to motivation.

Unexpectedly, expression of *Homer1*, *Zif268*, and *Arc* mRNAs were inherently lower in the NAc of LVR compared to WT rats (Fig. 4b–d control bars). Given the decreased inhibition on PKA that would stem from an endogenously low PKIα level in the NAc of LVR rats; we next tested whether the D1-like agonist SKF-38393 would reverse the lower IEG mRNA expression. D1 agonism caused endogenous *Zif268* and *Arc* mRNAs to have bi-directional changes between LVR and WT slices. *Zif268* and *Arc* mRNAs increased in LVR following D1 stimulation compared to their bilateral control levels, which was in contrast to WT, which favored to decrease IEG expression following D1 stimulation. We interpret the increased IEG expression following D1 stimulation as suggestive of increased sensitivity to dopamine signaling in LVR rats, possibly attributed to the decreased inhibitory role of PKIα in these rats. Fundamentally, activation of PKA in the NAc of rats has been shown to reduce the rewarding quality of cocaine [55]. Viral increases in CREB in the NAc shell of rats have revealed changes in reward-incentive motivation for cocaine [56]. Similarly, NAc overexpression of CREB has been shown to reduce the rewarding effects of sucrose, while expression of a dominant negative form of CREB had the opposite effect [57]. Taken together, we hypothesize that the contrasting IEG response to D1 agonism between WT and LVR rats may be indicative of differences in dopamine sensitivity and could, in part, underlie differing motivations to voluntarily run between WT and LVR rats.

The hypothesis is in some ways validated by the lack of difference in baseline IEG expression and in their evoked induction between virally overexpressed LVR and WT NAc slices (Fig. 4f–h). Interestingly, *Homer1* expression was decreased following 20 μM of SKF-38393 in LVR OV slices, which is strikingly similar to the lower expression in non-transfected WT slices following D1 stimulation compared to control levels (Fig. 4b). Taken together, PKIα overexpression had a normalizing outcome between LVR and WT rats, further suggesting the difference in IEG induction response to SKF-38393 in non-transfected rats could result from an endogenously low expression of PKIα in LVR rats.

PKIα Overexpression in Nucleus Accumbens Partly Rescues Low Voluntary Running in LVR Rats Bred to Run Low Distances

This is the first study, to the best of our knowledge, to address the NAc-specific role for the genetic manipulation of protein kinase inhibitor alpha (PKIα) on voluntary wheel-running behavior. Heretofore, our previous experiments (above) had established an association between PKIα mRNA levels and voluntary running phenotype. That being, our previous experiments showed; (1) PKIα mRNA in the nucleus accumbens (NAc) is lower in LVR than in WT, and (2) voluntary running distances is correlated when LVR and WT values were combined. From the above preliminary observations, the hypothesis was generated and tested that PKIα gene overexpression in NAc would increase nightly voluntary running distance in both LVR and WT.

PKIα Overexpression Responses in WT Were Generally in Contrary Direction to Those of LVR Rats

Unexpectedly, the examined molecular events in the nucleus accumbens were consistently dissimilar between LVR and WT to the same dosage of PKIα overexpression, as summarized in Table 2, and to be further described next. Firstly, a speculative interpretation is that WT rats attempted to resist further increases in voluntary running behavior, i.e., we hypothesize that presumed homeostatic mechanisms potentially

Table 2 Opposite directional changes in various parameters that were measured between low voluntary runners (LVR) and wild-type (WT) groups of rats. Up, horizontal, and down arrows indicate increase, no change, and decrease, respectively. From baseline, overexpression means rats previously had similar injection dosages of the rat PKIA gene into LVR and WT nucleus accumbens (NAc). Items 2–6 were measured in tissue punches quickly frozen in liquid nitrogen. Items 7–9 were determined in LVR and WT NAc in response to incubation of their NAc slices with 20 μm of the dopamine receptor 1 agonist SKF-38393, in either non-transfected slices or in those following PKIα overexpression. (* denotes trending directional change)

Wheel-running and molecular response to PKIα overexpression in LVR vs WT rats

Determination	LVR overexpression	WT overexpression
1. Voluntary running distance	↑	→
2. Total PKIα mRNA	→	↓
3. Endogenous PKIα mRNA	↑	→
4. NAc Drd1 receptor mRNA	→	↓
5. NAc Drd2 receptor mRNA	→	↓
6. NAc Fos mRNA	→	↓

Effect of SKF-38393 on IEG expression, non-transfected and PKIα overexpression in LVR vs WT rats

Determination	LVR non-transfected	WT non-transfected	LVR overexpression	WT overexpression
7. NAc Homer1 mRNA	↑	↓	↓	→
8. NAc Zif268	↑	↓	↓*	→
9. NAc Arc mRNA	↑	↓	→	→

regulated "sufficient" voluntary running distances prior to PKIα overexpression, and seemingly regulated against further increases in running distance. Second, LVR had a significant ~ 300% increase in voluntary running distance compared to empty-vector (EV) injected controls (Fig. 7c). However, WT rats had a non-significant ~ 15% difference from the EV group (Fig. 7c). In so, the same amount of PKIα gene that was locally injected into the NAc of WT, as LVR, failed to increase normal voluntary running distances (Fig. 7a, c). Third, different amounts of endogenous PKIα mRNA expression were present after equal viral-PKIα overexpression injections (Fig. 7e). WT rats had lower endogenous NAc expression of PKIα mRNA compared to endogenous PKIα expression in LVR rats (Fig. 7e). Fourth, WT had no significant difference in their total PKIα mRNA levels following overexpression, while LVR did (Fig. 7d). A brief interpretation could be that WT's NAc responds with a "ceiling" to running increases by viral overexpression of PKIα. Fifth, congruent with the effect of PKIα overexpression decreasing endogenous PKIα expression in WT rats, other transcripts related to dopamine signaling in the NAc were considered in hopes of explaining the lack of behavioral response in these rats [9]. Expression of Drd1, Drd2, and Fos mRNAs were lower in WT NAc punches following PKIα gene overexpression compared either to their EV control group or to LVR OV and EV groups (Fig. 7e, Table 2a). Collectively all of the above show unexpected, discordant directional changes following PKIα overexpression between LVR and WT rats (Table 2).

Though PKA has not been directly addressed in our experiments, previous studies have addressed D1 activity, which is positioned upstream of adenylyl cyclase and PKA, in the context of wheel-running behavior. Garland and Rhodes [38] revealed that systemic D1 antagonist SCH-23390 administration decreased wheel-running distance in control mice and in mice selectively bred to run high nightly distances. Further, Roberts et al. [24] determined that NAc-specific injections of both D1 agonist and antagonist decreased nightly wheel-running in high voluntary-running rats, but had no effect in low voluntary-running (LVR) rats. As hypothesized in the Roberts' study, this is likely reflective of differences in downstream signaling events in the NAc of LVR versus either HVR or WT rats. In line with observation addressing D1's agonism in the NAc on wheel-running behavior, increasing the activity of downstream target adenylyl cyclase was accomplished via foskolin, which, in turn, presumably increases PKA activity in the NAc. While WT rats decreased their nightly wheel-running behavior, LVR rats did not respond (Fig. 6). It has been suggested that D1 activity in the NAc receives phasic dopaminergic input, which acts to reinforce reward-incentive salient signals, a process that is overshadowed by tonic D1, and thus PKA, activity [58]. In so, the endogenously low expression of PKIα in LVR rats could speculatively reflect a naturally higher D1 input in the NAc and may, in part, explain the low motivation to voluntarily run in these rats.

In total, the above experiments highlight a promising gene candidate to partially rescue physical activity in a polygenic model of low voluntary running. In light of the overwhelming inactivity epidemic, these findings posit that PKIα acts as a neuro-molecular driver for the motivation to be physically active when voluntary running is less than normal in the selectively bred rats tested herein. Taken together, PKIα is a unique gene candidate to rescue physical inactivity in a polygenic model of low voluntary running.

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Compliance with Ethical Standards

Conflicts of Interest The authors declare that they have no conflict of interest.

References

1. Lavie CJ, Archer E, Nauman J (2017) Arrival and survival of the fittest. Am Heart J 196:153–155. https://doi.org/10.1016/j.ahj.2017.08.020.
2. Troiano RP, Berrigan D, Dodd KW et al (2008) Physical activity in the United States measured by accelerometer. Med Sci Sport Exerc 40:181–188. https://doi.org/10.1249/mss.0b013e31815a51b3
3. Ruegsegger GN, Booth FW (2017) Health benefits of exercise. Cold Spring Harb Perspect Med:a029694. https://doi.org/10.1101/cshperspect.a029694
4. den Hoed M, Brage S, Zhao JH et al (2013) Heritability of objectively assessed daily physical activity and sedentary behavior. Am J Clin Nutr 98:1317–1325. https://doi.org/10.3945/ajcn.113.069849
5. Roberts MD, Brown JD, Company JM, et al (2013) Phenotypic and molecular differences between rats selectively bred to voluntarily run high vs. low nightly distances. AJP Regul Integr Comp Physiol 304: R1024–R1035. doi: https://doi.org/10.1152/ajpregu.00581.2012
6. Ruegsegger GN, Toedebusch RG, Will MJ, Booth FW (2015) Mu opioid receptor modulation in the nucleus accumbens lowers voluntary wheel running in rats bred for high running motivation. Neuropharmacology 97:171–181. https://doi.org/10.1016/j.neuropharm.2015.05.022
7. Ruegsegger GN, Brown JD, Kovarik MC, Miller DK, Booth FW (2016) Mu-opioid receptor inhibition decreases voluntary wheel running in a dopamine-dependent manner in rats bred for high voluntary running. Neuroscience 339:525–537. https://doi.org/10.1016/j.neuroscience.2016.10.020
8. Knab AM, Bowen RS, Hamilton AT, Gulledge AA, Lightfoot JT (2009) Altered dopaminergic profiles: implications for the regulation of voluntary physical activity. Behav Brain Res 204:147–152. https://doi.org/10.1016/j.bbr.2009.05.034
9. Knab AM, Lightfoot JT (2010) Does the difference between physically active and couch potato lie in the dopamine system? Int J Biol Sci 6:133–150. https://doi.org/10.7150/ijbs.6.133
10. Mogenson GJ, Jones DL, Yim CY (1980) From motivation to action: functional interface between the limbic system and the motor system. Prog Neurobiol 14:69–97. https://doi.org/10.1016/0301-0082(80)90018-0
11. Berridge KC (2007) The debate over dopamine's role in reward: The case for incentive salience. Psychopharmacology 191:391–431. https://doi.org/10.1007/s00213-006-0578-x
12. Brené S, Bjørnebekk A, Åberg E, Mathé AA, Olson L, Werme M (2007) Running is rewarding and antidepressive. Physiol Behav 92: 136–140. https://doi.org/10.1016/j.physbeh.2007.05.015
13. Greenwood BN, Foley TE, Le TV et al (2011) Long-term voluntary wheel running is rewarding and produces plasticity in the mesolimbic reward pathway. Behav Brain Res 217:354–362. https://doi.org/10.1016/j.bbr.2010.11.005
14. Batty NJ, Fenrich KK, Fouad K (2016) The role of cAMP and its downstream targets in neurite growth in the adult nervous system. Neurosci Lett 652:1–8. https://doi.org/10.1016/j.neulet.2016.12.033
15. Nestler EJ (2016) Reflections on: "A general role for adaptations in G-proteins and the cyclic AMP system in mediating the chronic actions of morphine and cocaine on neuronal function". Brain Res 1645:71–74. https://doi.org/10.1016/j.brainres.2015.12.039
16. Beninger RJ, Miller R (1998) Dopamine D1-like receptors and reward-related incentive learning. Neurosci Biobehav Rev 22: 335–345. https://doi.org/10.1016/S0149-7634(97)00019-5
17. Roberts MD, Toedebusch RG, Wells KD, Company JM, Brown JD, Cruthirds CL, Heese AJ, Zhu C et al (2014) Nucleus accumbens neuronal maturation differences in young rats bred for low *versus* high voluntary running behaviour. J Physiol 592:2119–2135. https://doi.org/10.1113/jphysiol.2013.268805
18. Dalton GD, Dewey WL (2006) Protein kinase inhibitor peptide (PKI): a family of endogenous neuropeptides that modulate neuronal cAMP-dependent protein kinase function. Neuropeptides 40: 23–34. https://doi.org/10.1016/j.npep.2005.10.002
19. Chen X, Dai JC, Orellana SA, Greenfield EM (2005) Endogenous protein kinase inhibitor γ terminates immediate-early gene expression induced by cAMP-dependent protein kinase (PKA) signaling: Termination depends on PKA inactivation rather than PKA export from the nucleus. J Biol Chem 280:2700–2707. https://doi.org/10.1074/jbc.M412558200
20. Gangolli EA, Belyamani M, Muchinsky S, Narula A, Burton KA, McKnight GS, Uhler MD, Idzerda RL (2000) Deficient gene expression in protein kinase inhibitor alpha null mutant mice. Mol Cell Biol 20:3442–3448. https://doi.org/10.1128/MCB.20.10.3442-3448.2000
21. Pitts GC (1984) Body composition in the rat: interactions of exercise, age, sex, and diet. Am J Phys 246:R495–R501. https://doi.org/10.1152/ajpregu.1984.246.4.R495
22. Anantharaman-Barr HG, Decombaz J (1989) The effect of wheel running and the estrous cycle on energy expenditure in female rats. Physiol Behav 46:259–263. https://doi.org/10.1016/0031-9384(89)90265-5
23. Koch LG, Britton SL (2001) Artificial selection for intrinsic aerobic endurance running capacity in rats. Physiol Genomics 5:45–52
24. Roberts MD, Gilpin L, Parker KE, Childs TE, Will MJ, Booth FW (2012) Dopamine D1 receptor modulation in nucleus accumbens lowers voluntary wheel running in rats bred to run high distances. Physiol Behav 105:661–668. https://doi.org/10.1016/j.physbeh.2011.09.024
25. Hyatt HW, Toedebusch RG, Ruegsegger G, Mobley CB, Fox CD, McGinnis GR, Quindry JC, Booth FW et al (2015) Comparative adaptations in oxidative and glycolytic muscle fibers in a low voluntary wheel running rat model performing three levels of physical activity. Physiol Rep 3:e12619. https://doi.org/10.14814/phy2.12619
26. Grigsby KB, Kovarik CM, Rottinghaus GE, Booth FW (2018) High and low nightly running behavior associates with nucleus accumbens N-methyl-D-aspartate receptor (NMDAR) NR1 subunit expression and NMDAR functional differences. Neurosci Lett 671: 50–55. https://doi.org/10.1016/j.neulet.2018.02.011
27. Swallow JG, Carter PA, Garland T (1998) Artificial selection for increased wheel-running behavior in house mice. Behav Genet 28: 227–237. https://doi.org/10.1023/A:1021479331779
28. Eisenstein SA, Holmes PV (2007) Chronic and voluntary exercise enhances learning of conditioned place preference to morphine in rats. Pharmacol Biochem Behav 86:607–615. https://doi.org/10.1016/j.pbb.2007.02.002
29. Paxinos G, Watson C (1997) The Rat Brain in Stereotaxic Coordinates. Acad Press San Diego 3rd ed:
30. Zachor DA, Moore JF, Brezausek C, Theibert A, Percy AK (2000) Cocaine inhibits NGF-induced PC12 cells differentiation through

D1-type dopamine receptors. Brain Res 869:85–97. https://doi.org/10.1016/S0006-8993(00)02355-6
31. Jang J-H, Surh Y-J (2005) AP-1 mediates beta-amyloid-induced iNOS expression in PC12 cells via the ERK2 and p38 MAPK signaling pathways. Biochem Biophys Res Commun 331:1421–1428. https://doi.org/10.1016/j.bbrc.2005.04.057
32. Vyas DR, Spangenburg EE, Abraha TW, Childs TE, Booth FW (2002) GSK-3beta negatively regulates skeletal myotube hypertrophy. Am J Physiol Cell Physiol 283:C545–C551. https://doi.org/10.1152/ajpcell.00049.2002
33. Machida S, Spangenburg EE, Booth FW (2003) Forkhead transcription factor FoxO1 transduces insulin-like growth factor's signal to p27Kip1 in primary skeletal muscle satellite cells. J Cell Physiol 196:523–531. https://doi.org/10.1002/jcp.10339
34. Vasanwala FH, Kusam S, Toney LM, Dent AL (2002) Repression of AP-1 function: a mechanism for the regulation of Blimp-1 expression and B lymphocyte differentiation by the B cell lymphoma-6 protooncogene. J Immunol 169:1922–1929. https://doi.org/10.4049/jimmunol.169.4.1922
35. Meylan E, Dooley AL, Feldser DM, Shen L, Turk E, Ouyang C, Jacks T (2009) Requirement for NF-B signalling in a mouse model of lung adenocarcinoma. Nature 462:104–107. https://doi.org/10.1038/nature08462
36. Hoffmann HM, Nadal R, Vignes M, Ortiz J (2012) Chronic cocaine self-administration modulates ERK1/2 and CREB responses to dopamine receptor agonists in striatal slices. Addict Biol 17:565–575. https://doi.org/10.1111/j.1369-1600.2011.00353.x
37. Whishaw IQ, Cioe JDD, Previsich N, Kolb B (1977) The variability of the interaural line vs the stability of bregma in rat stereotaxic surgery. Physiol Behav 19:719–722. https://doi.org/10.1016/0031-9384(77)90304-3
38. Rhodes JS, Garland T (2003) Differential sensitivity to acute administration of Ritalin, apomorphine, SCH 23390, but not raclopride in mice selectively bred for hyperactive wheel-running behavior. Psychopharmacology 167:242–250. https://doi.org/10.1007/s00213-003-1399-9
39. Will MJ, Franzblau EB, Kelley AE (2004) The amygdala is critical for opioid-mediated binge eating of fat. Neuroreport 15:1857–1860. https://doi.org/10.1097/00001756-200408260-00004
40. Smith-Roe SL, Kelley AE (2000) Coincident activation of NMDA and dopamine D1 receptors within the nucleus accumbens core is required for appetitive instrumental learning. J Neurosci 20:7737–7742. doi: 20/20/7737 [pii]
41. Ruegsegger GN, Grigsby KB, Kelty TJ, Zidon TM, Childs TE, Vieira-Potter VJ, Klinkebiel DL, Matheny M et al (2017) Maternal western diet age-specifically alters female offspring voluntary physical activity and dopamine- and leptin-related gene expression. FASEB J 31:5371–5383. https://doi.org/10.1096/fj.201700389R
42. Willner P, Muscat R, Papp M (1992) Chronic mild stress-induced anhedonia: a realistic animal model of depression. Neurosci Biobehav Rev 16:525–534. https://doi.org/10.1016/S0149-7634(05)80194-0
43. Rygula R, Abumaria N, Flügge G, Fuchs E, Rüther E, Havemann-Reinecke U (2005) Anhedonia and motivational deficits in rats: impact of chronic social stress. Behav Brain Res 162:127–134. https://doi.org/10.1016/j.bbr.2005.03.009
44. Carr GD, Fibiger HC, Phillips AG (1989) Conditioned place preference as a measure of drug reward. In: The neuropharmacological basis of reward. Topics in experimental psychopharmacology. pp 264–319.
45. Flagel SB, Akil H, Robinson TE (2009) Individual differences in the attribution of incentive salience to reward-related cues: implications for addiction. Neuropharmacology 56:139–148. https://doi.org/10.1016/j.neuropharm.2008.06.027
46. Adami ANDREOLLO N, Freitas dos SANTOS E, Rachel ARAÚJO M, et al (2012) Rat's age versus human's age: what is the relationship? ABCD Arq Bras Cir Dig 25:49–51 . doi: https://doi.org/10.1590/S0102-67202012000100011
47. De Bartolomeis A, Tomasetti C (2012) Calcium-dependent networks in dopamine-glutamate interaction: the role of postsynaptic scaffolding proteins. Mol Neurobiol 46:275–296. https://doi.org/10.1007/s12035-012-8293-6
48. Duclot F, Kabbaj M (2017) The role of early growth response 1 (EGR1) in brain plasticity and neuropsychiatric disorders. Front Behav Neurosci 11. https://doi.org/10.3389/fnbeh.2017.00035
49. Girault JA (2012) Signaling in striatal neurons: the phosphoproteins of reward, addiction, and dyskinesia. Prog Mol Biol Transl Sci 106:33–62. doi: https://doi.org/10.1016/B978-0-12-396456-4.00006-7
50. Booth FW, Roberts CK, Thyfault JP, Ruegsegger GN, Toedebusch RG (2017) Role of inactivity in chronic diseases: evolutionary insight and pathophysiological mechanisms. Physiol Rev 97:1351–1402. https://doi.org/10.1152/physrev.00019.2016
51. Hill WF (1961) Effects of activity deprivation on choice of an activity incentive. J Comp Physiol Psychol 54:78–82. https://doi.org/10.1037/h0043817
52. Der-Avakian A, Markou A (2012) The neurobiology of anhedonia and other reward-related deficits. Trends Neurosci 35:68–77. https://doi.org/10.1016/j.tins.2011.11.005
53. Belke TW, Wagner JP (2005) The reinforcing property and the rewarding aftereffect of wheel running in rats: a combination of two paradigms. Behav Process 68:165–172. https://doi.org/10.1016/j.beproc.2004.12.006
54. Kalivas PW, Volkow ND (2005) The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatry 162:1403–1413. https://doi.org/10.1176/appi.ajp.162.8.1403
55. Self DW, Genova LM, Hope BT et al (1998) Involvement of cAMP-dependent protein kinase in the nucleus accumbens in cocaine self-administration and relapse of cocaine-seeking behavior. J Neurosci 18:1848–1859. https://doi.org/10.1523/JNEUROSCI.18-05-01848.1998
56. Carlezon WA, Thome J, Olson VG, et al (1998) Regulation of cocaine reward by CREB. Science (80-) 282:2272–2275. doi: https://doi.org/10.1126/science.282.5397.2272
57. Barrot M, Olivier JDA, Perrotti LI, DiLeone RJ, Berton O, Eisch AJ, Impey S, Storm DR et al (2002) CREB activity in the nucleus accumbens shell controls gating of behavioral responses to emotional stimuli. Proc Natl Acad Sci 99:11435–11440. https://doi.org/10.1073/pnas.172091899
58. Alleweireldt AT, Kirschner KF, Blake CB, Neisewander JL (2003) D1-receptor drugs and cocaine-seeking behavior: investigation of receptor mediation and behavioral disruption in rats. Psychopharmacology 168:109–117. https://doi.org/10.1007/s00213-002-1305-x