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Compulsive coping behaviour, which goal trackers do not develop, is exacerbated by chronic atomoxetine

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Keywords:

Schedule-Induced Polydipsia, compulsion, coping, rat, noradrenaline, nucleus accumbens

Word count:

6777 (incl. figure captions)

Abstract

Loss of control over coping strategies can result in the development of impulsive/compulsive spectrum disorders (ICSDs) such as obsessive-compulsive disorder or trichotillomania. Rats, like humans, show individual differences in their tendency to engage in and maintain control over coping behaviours. While most rats exposed to a schedule-induced polydipsia (SIP) procedure develop a controlled, moderate, coping polydipsic drinking response, some vulnerable individuals engage in excessive, compulsive drinking, or *hyper*dipsia. The development of hyperdipsia depends in part on noradrenergic mechanisms, as it is prevented by the noradrenaline reuptake inhibitor, atomoxetine in highly impulsive vulnerable individuals. However, whether noradrenergic mechanisms also underlie the expression of well-established hyperdipsia, or if other traits confer vulnerability to its development, are unknown. In two longitudinal studies in male Sprague Dawley rats, we investigated whether well-established hyperdipsia is influenced by atomoxetine and if its development is predicted by the ICSD-relevant sign-tracking trait. Sign-tracking predicted faster acquisition of adjunctive drinking but not the transition to hyperdipsia, while goal-tracking protected against the latter. Chronic atomoxetine exacerbated well-established hyperdipsia; post-mortem qPCR assays revealed that this was associated with increased cFos and zif268 mRNA, markers of cellular activity and plasticity, across the dorsal striatum. Atomoxetine also altered the transcriptomic landscape of the nucleus accumbens shell and the pattern of cFos and zif268 expression in the amygdalo-striatal system. These results provide new insights into the biobehavioural basis of compulsive behaviours, revealing a differential noradrenergic control of the development and the expression of compulsive coping.

Introduction

The ability to adaptively cope with stress and regulate negative emotions is paramount for resilience and wellbeing (Cabib et al., 2012; McLafferty et al., 2019; Moritz et al., 2016). However, some coping strategies become excessive and rigid, characteristic of psychiatric disorders such as mood/anxiety disorders (Matheson and Anisman, 2003) and impulsive/compulsive spectrum disorders (ICSDs) (Goodwin et al., 2014; Wood and Griffiths, 2007), including substance use disorders (SUDs) (Holt et al., 2013).

Adjunctive behaviours are a form of displacement behaviour expressed by many species as a means of coping with stress (Falk, 1971). An example of such coping behaviour is polydipsic drinking under a schedule-induced polydipsia (SIP) procedure, wherein food-deprived rats drink supra-homeostatic volumes of water in response to the stress induced by predictable, intermittent food delivery (Dantzer et al., 1988; Falk, 1961). Polydipsic drinking is anxiolytic, as it reduces plasma corticosterone levels (Dantzer et al., 1988; Levine et al., 1979; Velazquez-Sanchez et al., 2023) and anxiety-like behaviour (Belin-Rauscent et al., 2016; Marti-Prats et al., 2023), which are both increased by periodic food delivery in food-restricted individuals (Belin-Rauscent et al., 2016; Tazi et al., 1986).

While most rats maintain a stable, controlled level of polydipsia, some lose control over their coping response and develop maladaptive, excessive SIP drinking (Fouyssac et al., 2021; Merchan et al., 2019; Pellon et al., 2011). This *hyper*dipsia is compulsive in nature as it results in expending energy, whilst in a caloric deficit, to consume, warm, and excrete non-regulatory volumes of water (Flores et al., 2014; Moreno and Flores, 2012). While the acquisition of polydipsic coping behaviour depends on the mesolimbic dopaminergic system (Robbins and Koob, 1980), well-established compulsive hyperdipsia is controlled by the dopamine-dependent anterior dorsolateral striatum (aDLS) habit system (Marti-Prats et al., 2023). This shift in the neural locus of control is analogous to that accompanying compulsive drug-seeking habits and OCD-like repetitive behaviours (Everitt and Robbins, 2016; Robbins et al., 2024)

The biobehavioural factors contributing to individual differences in adaptive vs compulsive coping responses are not yet well understood. Motor impulsivity is associated with both the individual vulnerability to develop hyperdipsia and trait sign-tracking, i.e., the propensity to attribute incentive value to, and approach, reward-predictive conditioned stimuli (CSs) (Ansquer et al., 2014; Lovic et al., 2011; Schettino et al., 2022). At the neurochemical level, catecholaminergic mechanisms in the nucleus accumbens (NAc) underpin motor impulsivity, sign-tracking and the development of polydipsic drinking (Benn and Robinson, 2017; Economidou et al., 2012; Flagel et al., 2011; Fraser and Janak, 2017; Weissenborn et al., 1996). Yet, the neurochemical mechanisms underlying hyperdipsia appear to be distinct (Ansquer et al., 2014; Moreno et al., 2012; Velazquez-Sanchez et al., 2023). In particular, the development of hyperdipsia, is associated with transcriptional alterations in the noradrenergic locus coeruleus (LC) (Velazquez-Sanchez et al., 2023) and is prevented by the selective noradrenaline reuptake inhibitor atomoxetine, which also decreases impulsivity in hyperdipsia-vulnerable highly impulsive rats (Ansquer et al., 2014) characterised by heightened noradrenaline in the NAc (Moreno et al., 2010). It remains unclear whether noradrenergic mechanisms orchestrate the maintenance of well-established, aDLS dopamine-dependent habitual hyperdipsia or if they mediate the potential link between NAc catecholamine-dependent behaviours, like sign-tracking, and the development of compulsion.

In two longitudinal studies using male Sprague Dawley rats, we investigated if sign-tracking predicts an increased tendency to develop hyperdipsia, and whether well-established

hyperdipsia or sign-tracking is influenced by atomoxetine. Using post-mortem molecular biology assays, we also characterised the influence of atomoxetine on hyperdipsia-related cellular activity within the corticostriatal circuitry and expression patterns of candidate genes in the NAc shell (NAcS).

Experimental procedures

Subjects

One hundred and eight 10-week-old adult male Sprague-Dawley rats weighing between 280-355g at the start of the experiment were food restricted to 85-90% or 80-83% of theoretical free-feeding weight prior to autoshaping or SIP training, respectively. Water was freely available in the homecage. Two animals in Experiment 2 were excluded due to either excessive weight loss, despite intensive supportive care, or persistent emptying of the entire 300mL SIP water bottle. All procedures were conducted under the project license 70/8072 held by David Belin in accordance with the requirements of the UK Animals (Scientific Procedures) Act 1986, amendment 2012.

Experimental design

Experiment 1 (n=48) aimed to examine the relationship between sign-tracking and the propensity to develop polydipsic drinking or its compulsive manifestation, hyperdipsia. Rats were first assessed for their sign-tracking or goal-tracking tendency in an autoshaping task prior to being exposed to a SIP procedure, as described below and in the **Supplementary Online Materials** (**SOM**) (**Figure 1A**). Experiment 1 also aimed to assess the influence of acute manipulation of noradrenergic signaling on goal-tracking, sign-tracking, and polydipsic/hyperdipsic drinking via acute atomoxetine challenge following training on both autoshaping and SIP procedures (**Figure 1A**). Experiment 2 investigated, in a separate cohort (n=60), the behavioural, neural and cellular effects of chronic atomoxetine treatment on well-established, habitual aDLS dopamine-dependent hyperdipsia (Marti-Prats et al., 2023) – the same chronic treatment as previously shown to prevent the development of hyperdipsia in vulnerable highly impulsive rats (Ansquer et al., 2014) (**Figure 1B**.

Behavioural training and classification

The autoshaping task was conducted in twelve dedicated MedAssociates operant boxes as described previously (Vanhille et al., 2015) and detailed in the **SOM**. Briefly, an autoshaping session involved 25 unpredictable presentations of an 8s compound lever-light CS immediately followed by the delivery of a food pellet into a magazine. Contacts made with the lever (ie. sign-tracking behaviour) and entries into the magazine (ie. goal-tracking behavior) were recorded.

Rats were identified as sign-trackers (STs), intermediate performers (INT), or goal-trackers (GTs) using a K-means cluster analysis of sign-tracking and goal-tracking behaviours across the last three autoshaping sessions. This clustering approach, which allowed both types of behaviours to jointly and objectively inform classification, was set *a priori* to form three clusters (ST, INT, GT) based on previous work (Flagel et al., 2007; Jones et al., 2024).

Habituation, magazine training, and SIP training were carried out in twenty-four dedicated MedAssociates operant boxes as described previously (Ansquer et al., 2014) and detailed in the **SOM**. During the 60-min SIP sessions, one food pellet was delivered into the magazine on a fixed time 60s (FT60) schedule with water freely available in the operant box. Total water consumed (mL) throughout the session was measured.

Rats displaying high or low levels of polydipsic behaviour (high intermediate drinkers, IHD, and low intermediate drinkers, ILD, respectively), and those which demonstrated excessive, hyperdipsic, coping (high drinkers, HD) or very low, if any, polydipsic drinking (low drinkers, LD), were identified based on a quartile split of adjunctive water drinking over the last three SIP sessions. This approach maximizes differences between the extreme most populations, while reducing type I and preventing type II errors (Vanhille et al., 2015). Intermediate rats, which develop a controlled, polydipsic response, have hitherto seldom been considered in studies investigating the mechanisms of hyperdipsia (Fouyssac et al., 2021), often being amalgamated with the HD and LD groups (Mora et al., 2020; Pellon et al., 2011), thereby introducing type II errors and occluding our understanding of the mechanisms underlying adaptive polydipsic behaviour. Intermediate drinkers were split into ID and ILD subgroups to examine for the first time whether varying degrees of adaptive polydipsia are underpinned by distinct biobehavioural mechanisms.

Drugs

Atomoxetine hydrochloride (ATO, Sequoia Research Products, UK) was dissolved in sterile 0.9% saline vehicle. Atomoxetine (acute: 1.0, 3.0mg/kg; chronic: 1.0mg/kg; all at 1.0mL/kg) and vehicle were administered intraperitoneally (i.p) 30 minutes before behavioural sessions (Ansquer et al., 2014; Chernoff et al., 2021; Holden, 2022) as detailed in the **SOM**.

Molecular biology analyses

Forty-five minutes after the final SIP session, rats in Experiment 2 were anaesthetised under isoflurane and decapitated. Brains were rapidly extracted and flash frozen in -40°C isopentane. Coronal brain sections (300µm) were micro-punched (see Marti-Prats et al., 2021 and **SOM** for more details) from brain regions involved in impulsivity, compulsion and the associated transition from goal-directed to habitual control over behaviour: anterior and posterior insular cortex (aIC, pIC), nucleus accumbens shell and core (NAcS, NAcC), anterior dorsolateral striatum (aDLS), posterior dorsomedial striatum (pDMS), and basolateral and central amygdala (BLA, CeA) (Belin et al., 2013; Belin-Rauscent et al., 2016; Marti-Prats et al., 2023; Murray et al., 2015; Robbins et al., 2024). RNA was extracted and reverse transcribed into cDNA banks. Relative mRNA levels were determined in duplicate runs using qPCR (see Marti-Prats et al., 2021 and **SOM** for more details). Relative gene expression levels from the brain regions of interest were used to: 1) compare SIP-related cellular activity patterns between drinking (HD, LD) and treatment (atomoxetine, vehicle) groups using an immediate early gene (IEG) hotspot analysis (Magnard et al., 2024); 2) assess relationships between drinking behaviour and IEG activation across striatal and limbic regions as a proxy of drinking-related brain activation; and 3) conduct an exploratory network analysis to examine transcription patterns within IEG, catecholamine, and endogenous opioid systems of the NAcS – the only striatal subregion receiving notable noradrenergic innervation (Berridge et al., 1997; Delfs et al., 1998; Velazquez-Sanchez et al., 2023). Genes of interest and respective primers are listed in **Table S1**.

Data and statistical analyses

Data, presented as mean ± SEM and/or individual data points, were analysed using SPSS 28.0.1 (IBM Corporation, USA) as detailed in the **SOM**. Assumptions of normality, homogeneity of variance, and sphericity were tested using Shapiro-Wilk, Levene, and Mauchley tests, respectively. In instances where normality was violated, as for a few genes in the qPCR experiment, data were log-transformed before analysis. If sphericity was violated, the Huynh-Feldt correction was applied. The relative distribution of subjects across SIP and autoshaping groups were compared using a Chi-squared test and z-test of independent proportions. Behavioural data were subjected to Analyses of Variance (ANOVA) with group (ST vs GT, or

HD, IHD, ILD, LD) and/or treatment (vehicle vs atomoxetine) as between-subject factors, and time (daily sessions) as the within-subject factor (**SOM)**. On confirmation of significant main effects or interactions, differences among individual means were further analysed using Sidak-corrected post-hoc comparisons. Effect sizes for ANOVA effects and pairwise comparisons were reported as partial eta squared (η^2) and Cohen's d, respectively. Multivariate and simple linear regression was used to assess the extent to which variation in performance on the autoshaping task or SIP could predict behaviour on the other paradigm (**SOM**). For the hotspot analysis, univariate ANOVA with SIP group and treatment as between-subject factors was used to compare IEG mRNA levels between groups for the compulsion-relevant brain regions listed above. Relationships between behavioural and molecular variables were examined using Spearman correlations (**SOM**). Transcription networks containing gene-gene Spearman correlations which survived Benjamini-Hochberg correction were used to visually explore and describe transcription patterns within the NAcS of vehicle- or atomoxetine-treated HD and LD rats (**SOM**). Figures were generated using GraphPad Prism 10.2 (GraphPad, USA) and Adobe Illustrator (Adobe, CA, USA), and network plots were created using Cytoscape 3.10 (Institute for Systems Biology, USA).

Results

Experiment 1:

Sign tracking predicts the tendency to engage in high, but not compulsive, levels of coping behaviour.

In the autoshaping task, distinct groups were revealed by k-means clustering based on ST and GT approach behaviours (**Figure 2A**). STs made more CS-concurrent lever contacts than GTs [session x group: $F_{8,180}$=17.240, p<0.001, η^2=0.434; GTs vs STs- all sessions: Sidak ps≤0.003, ds≥0.504] (**Figure 2B**), which instead entered the food magazine more than the former during CS presentation [session x group: $F_{8,180}$=23.766, p<0.001, η^2=0.514; GTs vs STs- session 1: Sidak p=0.094; sessions 2-5: Sidak ps≤0.001, ds≥1.165] (**Figure 2B)**. After more than three weeks of exposure to a SIP procedure, these rats were shown to engage in different degrees of coping behaviour. HD rats exhibited compulsive coping, or hyperdipsia, with much higher levels of polydipsic drinking than IHD and ILD rats, which demonstrated high or low levels of adaptive coping behaviour, and LD rats, which did not readily develop SIP with water, as previously reported (Fouyssac et al., 2021; Marti-Prats et al., 2023) (**Figure 2C**). HD rats quickly lost control over their coping responses, since they drank more than IHD, LHD and LD rats from the 8th, 6th, and 3rd SIP session onward, respectively [session x group: $F_{51,782}$=3.402, p<0.001, η^2=0.188; HD vs ILD- sessions 1-5: Sidak ps≥0.087; sessions 6-18: Sidak ps≤0.036, ds≥0.416; HD vs IHD- sessions 1-7: Sidak ps≥0.188; sessions 8-18: Sidak ps≤0.012, ds≥0.475; HD vs LD- sessions 1-2: Sidak ps≥0.770; sessions 3-18: Sidak ps≤0.042, ds≥0.408] (**Figure 2D**). IHD and ILD rats differed from LDs in their coping response from session 12 and 13 onwards [IHD vs LD- sessions 1-11: Sidak ps≥0.085; sessions 12-18: Sidak ps≤0.003, ds≥0.545; ILD v LD- sessions 1-12: Sidak ps≥0.534; sessions 13-18: Sidak ps≤0.054, ds≥0.393], respectively, while differences in polydipsic drinking between them emerged after the 14th session [IHD vs ILD- sessions 1-14: Sidak ps≥0.152; sessions 15-18 ps≤0.037, ds ≥ 0.393] (**Figure 2D**).

GT/ST rats were asymmetrically distributed across SIP groups [$\chi^2_{3,n=19}$ = 10.795, p = 0.013]. There was a profound enrichment of ST rats in the HD and IHD groups, with the exception of a single HD GT rat, whereby the IHD group contained only ST rats [IHD- ST vs GT: z=-1.920, p=0.055]. In contrast, the ILD group contained exclusively GT rats [ILD- GT vs ST: z=-3.055, p=0.002] (**Figure 2E**) and there were more GT rats in the ILD group than any other SIP group

[GTs- ILD vs HD: z=-2.582, p=0.010; ILD vs IHD: z=-3.000, p=0.003; ILD vs LD: z=-2.070, p=0.038; all other |zs|≤1.920, ps≥0.055] (**Figure 2E**).

GTs and STs also differed in the speed at which they acquired polydipsic drinking. STs drank significantly more water than GTs during early SIP (d1-9) [group x block x session: $F_{8,136}$=2.722, p=0.008, η^2=0.138; early block- session x group: $F_{8,136}$=2.375, p=0.020, η^2=0.123; GTs vs STs- sessions 1,2,7,8: Sidak ps≤0.033, ds≥0.335; sessions 3-6: Sidak ps≥0.057, ds≤0.294]. (**Figure 3A**), consistent with the relatively greater proportion of GTs in lower SIP groups (ILD, LD) (**Figure 2E**). As SIP became more established, GTs eventually reached comparable levels of polydipsic drinking to STs such that water consumption during later training sessions (d10-18) was no longer distinguishable between the groups [late block- session x group interaction: $F_{8,136}$=1.330, p=0.234]. (**Figure 3A**). Rats which developed hyperdipsia (HD) did not differ from non-polydipsic LD rats in goal-tracking magazine entries [session x group: $F_{12,176}$=3.968, p<0.001, η^2=0.213; all sessions Sidak ps≥0.413] nor in sign-tracking lever presses [session x group: $F_{12,176}$=2.949, p=0.007, η^2=0.167; HD vs LD: all sessions Sidak ps≥0.485] (**Figure 3B**). However, ILD rats made fewer sign-tracking lever contacts [ILD vs IHD- sessions 3-5: Sidak ps≤0.027, ds≥0.431; sessions 1-2: Sidak ps≥0.515; ILD vs HD- sessions 2-5: Sidak ps≤0.048, ds≥0.400; session 1: Sidak p=0.089] and more goal-tracking magazine entries [ILD vs IHD- sessions 3-5: Sidak ps≤0.030, ds≥0.426; session 1-2: Sidak ps≥0.598; ILD vs HD- sessions 3-5: Sidak ps≤0.040, ds≥0.410; sessions 1-2: Sidak ps≥0.080] than both IHD and HD rats (**Figure 3B**), thereby indicating that Pavlovian conditioned approach tendencies are expressed to the lowest degree in the group of rats which adopted a controlled adaptive coping strategy. Goal-tracking and sign-tracking of adaptive ILD rats did not significantly differ from that of the LD group [CS lever presses: all sessions Sidak ps≥0.146; CS magazine entries: all sessions Sidak ps≥0.110] (**Figure 3B**).

A multivariate linear regression model that was used to estimate the extent to which the level of adjunctive drinking could be predicted by both goal-tracking and sign-tracking did not yield any statistically significant result in any of the SIP phenotypes (HD: $F_{2,9}$=1.980, p=0.194; IHD: $F_{2,9}$=0.383, p=0.693; ILD: $F_{2,9}$=3.052, p=0.097; LD: $F_{2,9}$=0.297, p=0.769). However, simple linear regression revealed that goal-tracking magazine entries alone predicted the level of adjunctive drinking in the ILD group [ILD: $F_{1,10}$=6.462, p=0.029; HD: $F_{1,10}$=2.943, p=0.117; IHD: $F_{1,10}$=0.371, p=0.556; LD: $F_{1,10}$=0.040, p=0.845, R^2=0.393; average ILD water consumption = 10.032 + 0.017*(average CS magazine entries)] (**Figure 3C-D**). Thus, while goal-tracking accounted for 39.3% of the variance in adjunctive drinking in ILD rats (**Figure 3C**), it did not predict their baseline, regulatory drinking (R^2=0.003, p=0.863), and sign-tracking alone predicted neither the level of regulatory drinking (R^2=0.000, p=0.962) nor that of adjunctive drinking for any SIP group (all R^2s≤0.148, ps≥0.217) (**Figure 3E-F**).

Acute atomoxetine treatment does not influence Pavlovian approach or polydipsic coping.

Acute atomoxetine administration did not influence sign-tracking [Fs≤0.237, ps≥0.880] (**Figure 4A**) but it resulted in a slight group-dependent decrease in goal tracking [dose x group: $F_{4,76}$=3.160, p=0.019, η^2=0.143] (**Figure 4B**). The high dose of atomoxetine tended to decrease magazine entries in GTs [GTs- 3.0 vs vehicle: Sidak p=0.055, d=0.354] (**Figure 4B**), while the low dose reduced this behaviour in ILD rats [ILD- 1.0 vs vehicle: Sidak p=0.002, d=0.536] (**Figure 4B**), characterised by a particularly high level of goal-tracking (see **Figure 3D**). Consequently, the effect of atomoxetine on goal-tracking in ILD rats no longer reach statistical significance when baseline magazine entries were used as a co-variate in the above analysis [group x dose: $F_{6,74}$=2.240, p=0.060; ILD- 1.0 vs vehicle: Sidak p=0.372] (**Figure 4B**).

Acute administration of atomoxetine did not significantly affect either the well-established expression of adaptive or compulsive coping behaviour [dose: $F_{2,76}$=2.911, p=0.061, η^2=0.071;

dose x group: Fs≤2.301, ps≥0.096;] (**Figure 4C**) in marked contrast with the effect of a chronic administration of the drug on either the development of hyperdipsia (see Ansquer et al., 2014) or its expression, as shown below.

Experiment 2

Chronic atomoxetine treatment exacerbates compulsive coping behaviour.

As in Experiment 1, individual differences in adjunctive coping behaviour appeared after three weeks of exposure to a SIP procedure in a separate cohort of rats. HD rats in Experiment 2 lost control over their coping behaviour, drinking significantly more than LD (**Figure 5A&C**), ILD, and IHD rats (**Figure 5C&D**) from session 2, 11, and 18, respectively [session x group: $F_{60,980}$=4.952, p<0.001, η^2=0.408; HD vs LD- session 1: p=0.066; sessions 2-21: ps≤0.054, ds≥0.392; HD vs ILD- sessions 1-10: ps≥0.081; sessions 11-21: ps≤0.029, ds≥0.380; HD vs IHD- sessions 1-17: ps≥0.173; sessions 18-21: ps≤0.043, ds≥0.300]. IHD rats demonstrated greater water drinking than LD (**Figure 5C&D**) and ILD (**Figure 5B&D**) rats from day 9 and 12 onward [IHD vs LD- sessions 1-8: p≥0.073; sessions 9-21: ps≤0.007, ds≥0.445; IHD vs ILD- sessions 1-11: Sidak ps≥0.138; sessions 12-21: ps≤0.044, ds≥0.403]. The difference in water consumption between ILD and LD rats emerged after session 19 [ILD vs LD- sessions 1-19: Sidak ps≥0.140; sessions 20-21: ps≤0.034, ds≥0.382] (**Figure 5C&D**).

After twenty-one SIP sessions, at a time the control over coping behaviour has devolved in the aDLS dopamine-dependent habit system (Marti-Prats et al., 2023), HD, IHD, ILD and LD rats were each randomly assigned to receive vehicle or atomoxetine administration (1.0mg/kg, i.p.) prior to each daily SIP session for 17 days, with groups matched for baseline water consumption (**Figure 5A&B**). Chronic atomoxetine administration selectively exacerbated hyperdipsia as revealed by a substantial increase in the degree of adjunctive drinking in HD rats, compared to vehicle-treated HD controls [group x treatment x block: $F_{9,147}$=3.656, p=0.003, η^2=0.183; HD: atomoxetine vs vehicle- early treatment block: Sidak p=0.001, d=0.500; late treatment block: Sidak p=0.012, d=0.380] (**Figure 5C**), with no effect on drinking in LD (**Figure 5C**), IHD or ILD (**Figure 5D**) rats [atomoxetine vs vehicle- early treatment block: all Sidak ps≥0.117; late block: all Sidak ps≥0.350].

Molecular correlates of the exacerbation of compulsive coping by atomoxetine

Immediate early gene (IEG) hotspot analysis

Chronic atomoxetine treatment increased cFos mRNA levels in both the aDLS [treatment: $F_{1,27}$=6.881, p=0.014, η^2=0.203] and the pDMS [treatment: $F_{1,27}$=4.913, p=0.035, η^2=0.154] (**Figure 6A**), while it decreased zif268 mRNA levels in the former [treatment: $F_{1,27}$=5.347, p=0.029, η^2=0.165] (**Figure 6B**) in all rats. No differences between compulsive and non-compulsive rats (see **SOM**) and no other effects of atomoxetine were found in any other region of interest [Fs≤3.668, ps≥0.066] (**Figure 6C&D**).

In vehicle-treated rats, irrespective of drinking group, the level of water intake was associated with zif268 mRNA levels in the aDLS [rho=0.524, p=0.045] and the NAcC [rho=0.596, p=0.019] (**Figure 6E&F**), while compulsive water drinking in vehicle-treated HD rats was specifically associated with zif268 mRNA levels in the NAcS [rho=0.788, p=0.035; all other groups: ps≥0.643] (**Figure 6G**). This relationship was absent in atomoxetine-treated HDs rats in which a negative correlation between hyperdipsic drinking and cFos mRNA levels emerged in the NAcC alongside a negative relationship between drinking and cFos mRNA levels in the CeA that were not present in their vehicle-treated counterparts [rho=-0.762, p=0.028 and rho=0.690, p=0.058, respectively], respectively (**Figure 6H&I**). Neither NAcC or CeA cFos levels significantly

correlated with water consumption in LDs nor in vehicle-treated HDs (**Figure 6J&K**) and no other significant correlations were observed [ps≥0.094].

Effect of chronic atomoxetine on the compulsion-related NAcS transcriptional landscape

Within the NAcS, where zif268 mRNA was selectively related to compulsive water drinking (**Figure 6G**), the mRNA levels of each gene of interest did not differ between vehicle- or atomoxetine-treated HD and LD rats [Fs≤2.304; ps≥0.146] (**Supplementary Figure 1A-B**). In vehicle-treated HD rats, mRNA levels of the noradrenergic receptors (NArs) did not tend to correlate with cFos and zif268 (**Figure 6M**), in marked contrast to the pattern observed in LD rats (**Figure 6L**). Indeed, in vehicle-treated LD rats, cFos and zif268 mRNA levels were closely related to those of several NArs, in a system where all NAr mRNA levels were highly correlated to each other (**Figure 6L**). These compulsion-specific NAcS transcriptional relationships were profoundly altered by atomoxetine (**Figure 6N-O**). In LD rats, atomoxetine resulted in a loss of many NAr-NAr and NAr-IEG correlations, accompanied by emergent kappa/dynorphin-D1 dopamine receptor and D2-Dar-zif268 mRNA correlations (**Figure 6N**). Atomoxetine-treated HD rats showed emergent correlations between DArs and IEGs mRNA levels at the expense of those observed between all three β NAr subtypes in their vehicle-treated counterparts (**Figure 6O**). All Spearman rho values are presented in **Supplementary Table 2**.

Discussion

The present results reveal that, at the population level, sign-tracking facilitates the acquisition of coping behaviour manifested as adjunctive drinking, while goal-tracking protects against the transition to excessive or compulsive coping, manifested as hyperdipsia (Marti-Prats et al., 2023; Velazquez-Sanchez et al., 2023). In addition, goal-tracking predicts the magnitude of polydipsic drinking in a subgroup of rats that adopt an adaptive coping strategy.

In contrast to the protective effect that chronic (but not acute) atomoxetine treatment has against the development of hyperdipsia in vulnerable highly impulsive rats (Ansquer et al., 2014), here it selectively exacerbated hyperdipsia once behaviour was well-established and mediated by the aDLS dopamine-dependent habit system (Marti-Prats et al., 2023). At the neural systems level, atomoxetine abolished the compulsion-specific relationship between drinking behaviour and activity-related plasticity in the NAcS, and engaged cellular activity across the dorsal striatum. Atomoxetine decreased mRNA levels of the plasticity marker zif268 (Knapska and Kaczmarek, 2004), indicating a reduction in activity-related plasticity in the aDLS. Chronic atomoxetine also disrupted the pattern of SIP-related plasticity in the incentive habit-related NAcC-aDLS circuit (Belin et al., 2013), the activation of which was associated with the magnitude of adjunctive drinking in vehicle controls, regardless of its adaptive or compulsive nature. These are intriguing effects, considering that only the NAcS receives noradrenergic innervation (Berridge et al., 1997; Delfs et al., 1998; Schroeter et al., 2000; Velazquez-Sanchez et al., 2023).

Within the NAcS, zif268 mRNA levels were uniquely related to the severity of hyperdipsia. An exploratory transcriptional network analysis indicated that compulsive HD rats exhibited less transcriptional convergence within the NAcS (i.e., fewer correlations) between mRNA levels of IEGs and candidate genes of the catecholamine and endogenous opioid systems, than LD rats. In the former, chronic atomoxetine treatment further decreased intra-NAcS correlations between mRNA levels of noradrenaline receptors and IEGs in favour of emergent correlations between DA receptor and IEG mRNA levels. This suggests a switch from a noradrenergic to a dopaminergic dominance in the catecholaminergic influence on coping-related NAcS cellular activity and plasticity.

Both the acquisition of polydipsia under SIP and sign tracking depend on catecholaminergic mechanisms in the NAc (Fraser and Janak, 2017; Robbins and Koob, 1980; Saunders and Robinson, 2012). These overlapping neurochemical mechanisms may explain the facilitated development of adjunctive drinking observed in ST rats. The differences between ST and GT rats in their coping response progressively subsided, eventually disappearing when coping had become well-established and habitual (Marti-Prats et al., 2023). Thus, the differences in coping behaviour between STs and GTs are restricted to the speed of acquisition, which may be accounted for by their differential behavioural response to Pavlovian cues. One hypothesis for future research is that the SIP chamber itself may act as an occasion setter for the negative reinforcing properties of the drinking-induced anxiolysis that accompanies the development of polydipsic drinking (Marti-Prats et al., 2023; Tazi et al., 1986), which may be differentially imbued with incentive salience by ST and GT rats (Fraser and Janak, 2019). Since ST rats form Pavlovian associations more readily than GT rats (Flagel et al., 2009; Keefer et al., 2020), a higher incentive value ascribed to the chamber may promote the anxiolytic drinking response via Pavlovian-to-instrumental transfer (PIT) early on in training in these animals (Cartoni et al., 2013; Fraser and Janak, 2019; Holmes et al., 2010).

Goal-tracking was instead associated with the tendency to develop low levels of polydipsic coping in intermediate drinking rats. In these ILD rats, goal-tracking correlated with the magnitude of the adjunctive strategy, a relationship not found in hyperdipsic, compulsive HD nor in non-polydipsic LD rats. Thus, goal-tracking may predict the predilection toward moderate and adaptive coping strategies. This is in line with recent evidence showing that stronger goal-tracking tendencies relate to reduced alcohol consumption in rats (Hakus et al., 2025).

Goal-tracking behaviour was reduced specifically in GTs by acute atomoxetine administration. This effect is only partially in agreement with the previous demonstration that acute atomoxetine influences both sign-tracking and goal-tracking (Holden, 2022). Several methodological differences may explain these discrepancies, such as the much younger age at which animals in the present study were characterised (70 vs ~100 days, respectively), or the way in which GT and ST groups were defined. The previous study used a tripartite Pavlovian conditioned approach index to classify STs and GTs (Holden, 2022) rather than a k-means clustering algorithm applied to the sign- and goal-tracking behaviours (CS-lever contacts and CS-magazine entries). Together with a tendency for younger individuals to display higher sign-tracking tendencies (DeAngeli et al., 2017), the objective, data-driven classification approach adopted in the present study may result in the identification of a smaller population of ST rats (22% vs 40%) characterised by a more extreme phenotype (~90 CS-lever contacts per session vs ~65), potentially less pervious to acute atomoxetine administration. Acute atomoxetine also had no effect on well-established coping behaviour under SIP, thereby suggesting that a chronic treatment, such as used in Experiment 2, and the consequent neuroplastic adaptations, are necessary to influence habitual, aDLS DA-dependent compulsive coping behaviour.

Indeed, chronic atomoxetine treatment exacerbated well-established hyperdipsia in HD rats, opposite to its effect on the development of hyperdipsia in vulnerable highly impulsive rats under the exact same SIP conditions (Ansquer et al., 2014). Taken together these results demonstrate that neuroplastic mechanisms engaged by protracted engagement in coping behaviour across the development of compulsion in vulnerable individuals are associated with profound alterations in noradrenergic signalling. This is in agreement with evidence that the development of hyperdipsia is accompanied by a downregulation of the plasticity-related gene Arc in a subset of LC neurons in HD rats (Velazquez-Sanchez et al., 2023). Therefore, while non-vulnerable rats may be able to engage adaptive plasticity mechanisms during the development of ventral striatum dopamine-dependent polydipsic drinking to maintain control over their coping behaviour, vulnerable HD rats cannot do it without potentiation of noradrenergic signalling by

atomoxetine. The aberrant plasticity within noradrenergic systems that emerges with protracted coping in HD rats (Velazquez-Sanchez et al., 2023) results in a diametrically opposite control over behaviour between the acquisition of adjunctive coping and its manifestation as an aDLS dopamine-dependent compulsive habit. Further research is warranted to elucidate the precise mechanisms of this shift. However, the distinct effects of repeated atomoxetine on the development of ventral striatum dopamine-dependent polydipsic drinking and the expression of aDLS dopamine-dependent hyperdipsia demonstrates the key role of noradrenergic mechanisms in the control of hyperdipsia via at least two corticostriatal circuits involved in compulsive coping behaviour (Robbins et al., 2024).

Chronic exposure to atomoxetine can induce enduring functional changes via neuroplastic alterations within the ventral striatal catecholamine system (Ruocco et al., 2010) and other corticolimbic networks (Fumagalli et al., 2010; Sun et al., 2012). At the cellular level, the effects of chronic atomoxetine treatment can be mediated by activation of pre-synaptic α2-autoreceptors, which would result in a reduction in spontaneous LC neuronal firing and noradrenaline release (Bari and Aston-Jones, 2013) or alterations in the expression or transduction of specific postsynaptic noradrenergic and other monoaminergic receptors in response to chronically elevated extracellular NA levels (Ruocco et al., 2010). Coupled with dysregulated plasticity within the noradrenergic LC of compulsive rats, such adaptations in the NAcS may contribute to the exacerbation of hyperdipsia by atomoxetine through remote control over aDLS dopamine-dependent mechanisms underlying compulsive behaviours (Belin et al., 2008 ; 2011; 2010; Haber et al., 2000) via the ascending dopamine-dependent spiralling circuitry (Ikemoto, 2007).

The NAcS transcription patterns explored in the present study start shedding light on such compulsion-specific adaptations in the mesolimbic system and their alteration by atomoxetine. Compared to vehicle-treated LD rats in which mRNA levels of every NAr and DAr subtype were correlated with at least two other catecholamine receptors, vehicle-treated HDs exhibited very little convergence in the level of expression of NArs and DArs, suggesting a compulsion-specific divergence in the regulation of post-synaptic monoaminergic receptors. Additionally, vehicle-treated HDs also showed few correlations between NArs and DArs and the two markers of cellular activity and plasticity, cFos and zif268. This is in marked contrast to the very high convergence of mRNA levels of both IEGS and those of NArs and DArs observed in the NAcS of vehicle-treated LDs, whereby five catecholamine markers correlated with zif268 and two NArs correlated with cFos. Together, this indicates an uncoupling of NAr-related mechanisms from those associated with cellular activity and plasticity in the NAcS in compulsive individuals, as opposed to the shared involvement of noradrenergic and dopaminergic mechanisms observed in LD rats. Such alterations in compulsive rats are consistent with the evidence for the independent, but interacting, role the two catecholamines play in compulsive coping and compulsion vulnerability traits like impulsivity (de Villiers et al., 1995; Moreno et al., 2010; Russell et al., 2000; Russell, 2000; Velazquez-Sanchez et al., 2023) .

Interestingly, atomoxetine resulted in a similar disruption of correlations between NAr and IEG mRNA levels in the NAcS in all rats, demonstrating the ability of chronic noradrenergic pharmacological manipulation to influence the relationship between NAr expression in and the functional engagement (activation and activity-related plasticity) of the NAcS (Filipkowski et al., 2006; Jones et al., 2001). In compulsive rats, atomoxetine-exacerbated hyperdipsia was accompanied by emerging correlations between the mRNA levels of DArs and those of cFos and zif268, suggesting a drug-induced functional coupling between dopaminergic mechanisms and those mediating the functional engagement of the NAcS. One may hypothesise that this reflects a treatment-induced re-engagement of the DA-dependent NAc activity which otherwise underlies SIP acquisition (Mittleman et al., 1990; Robbins and Koob, 1980). At a time where

coping responding is dependent on the aDLS dopamine-dependent habit system, such putative reengagement of ventral striatal dopamine-dependent motivational processes may contribute to an increased vigor of the compulsive habit (Belin et al., 2013). In this context, given that dopaminergic mechanisms in the ventral striatum influence the functional engagement of both the pDMS and the aDLS (O'Doherty et al., 2004; Yin et al., 2008), the atomoxetine-induced increase in cFos across both dorsal striatal territories observed is not surprising. Indeed, while neither dorsal striatal territory receives noradrenergic innervation (Velazquez-Sanchez et al., 2023), they are both under functional control of catecholaminergic mechanisms in the NAcS via spiralling striato-meso-striatal connections (Ikemoto, 2007). In addition, aDLS DA-dependent maladaptive habits are also maintained by functional interactions with the CeA (Murray et al., 2015), which was shown here to be recruited by atomoxetine in HD rats, as revealed by the emergent correlation between hyperdipsic drinking and CeA cFos expression. Atomoxetine treatment also blunted SIP-related plasticity (as assessed by zif268 mRNA levels) within the incentive habit system (namely NAcC-aDLS) (Belin et al., 2013), which otherwise tracked adjunctive drinking in vehicle-treated controls. Together these data suggest that chronic atomoxetine treatment aberrantly engaged dopamine-dependent motivational mechanisms within a rigid incentive habit system, originating in the ventral striatum, thereby exacerbating compulsion in HD rats.

It is important to acknowledge the exploratory and descriptive nature of the transcriptional network analysis in Experiment 2. Thus, the interpretations coming from these transcriptomic pattern analyses offer insight into the overall transcriptional signature of atomoxetine-invigorated compulsion and provide a theoretical framework from which future lines of mechanistic experiments can be derived.

Nevertheless, these data reveal a novel relationship between goal-tracking and controlled polydipsic coping, and further elucidate the differential contribution, and associated transcriptional signature, of noradrenergic mechanisms in the development vs the expression of compulsive coping, mediated by the ventral and dorsolateral striatum, respectively. These data also provide novel insights into the mechanisms by which atomoxetine induces or worsens compulsions in humans (Araz Altay, 2019; İnce et al., 2015).

Data availability statement:

The data can be available at: link to Cambridge repository will be added upon acceptance.

Authors contributions:

DB and ABR designed the experiments. ABR, AHM, ST, BN, CY and MP carried out the behavioural experiments. ABR and MF carried out the molecular biology experiments. CC, SJ and DB analysed and interpreted the data and wrote the manuscript.

Acknowledgements and funding:

This work, carried out at the Department of Psychology of the University of Cambridge, was funded by UKRI grants to DB (MR/W019647/1 and (MR/N02530X/1 (PI: Barry Everitt), a Leverhulme Trust Early Career (ECF-2018-713) and Isaac Newton Trust fellowship (18.08(g)) to MF and Cambridge Trust International Scholarship (10712081 - 2023) and NSERC Postgraduate Scholarship – Doctoral (PGSD3 - 579465 – 2023) to CC. The authors would like to thank the technical staff of the University Biomedical Services of the Combined Facility for the husbandry and care provided to the animals involved in this research.

Competing interests:

The authors declare no competing financial interests.

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Figure legends

Figure 1. Timeline of the experiments

A) Following 7 days of habituation after arrival at the facility, rats were food restricted to 85-90% of their theoretical free-feeding weight. Across the three consecutive days prior to autoshaping training, rats underwent habituation to sugar pellets and the autoshaping chamber, and basic magazine training. Next, rats were trained on the autoshaping task (AS) for five sessions, after which the atomoxetine challenge began according to a counterbalanced Latin square design (see SOM for details). Rats were then further food restricted to 80-85% of their theoretical free-feeding weight, and habituated to the SIP chamber and the fixed time 60-second (FT-60) food pellet delivery schedule. SIP training was then performed for 18 60-min sessions. The second atomoxetine challenge occurred according to the schedule as for the previous Latin square. **B)** In Experiment 2, following acclimatization to the facility, rats were food-restricted to 80-85% of theoretical free-feeding weight. During the last two days of food restriction, rats underwent habituation and magazine training as in Experiment 1. Rats were then performed SIP for 21 days, during which high, high intermediate, low intermediate (ie. adaptive) and low drinkers were identified. Equal numbers of rats from each drinking group then received intraperitoneal (i.p.) injections of atomoxetine (1mg/kg) or vehicle 30 mins before each daily SIP session for 17 days. Forty-five minutes after the final SIP session, rats were sacrificed and their brains were harvested and flash frozen for subsequent qPCR analyses.

Figure 2. Characterization of individual differences in sign-tracking, goal-tracking and polydipsic coping behaviour.

A) A k-means clustering algorithm was used to categorize rats as sign trackers (STs), goal trackers (GTs), or intermediate performers (INT) based on average lever presses and magazine entries made during the CS-presentation across the last three autoshaping training sessions. The "X"s represent cluster centres. **B)** As expected, STs and GTs demonstrated greater responding either on the lever or at the food magazine, respectively, during the CS presentation. **C)** Rats were defined as high (HD), high intermediate (IHD), low intermediate (ILD), or low (LD) drinkers based on a quartile split of average water consumption across the last 3 days of SIP training. **D)** Differences between SIP groups emerged at various points in acquisition, such that by the end of SIP training, groups exhibited distinct levels of water consumption across the last three SIP training sessions (BL)**.** **E)** Across SIP groups, there was a disproportionate representation of GTs and STs, such that there were fewer STs vs GTs in the ILD group. There were also significantly more GTs in the ILD group versus any other SIP group. Data are presented as A) individual datapoints representing each subject, B-D) group means ± SEM, or E) number of subjects per group. Symbols in C) indicate autoshaping group (GT = inverted triangle; ST = triangle). ***significant Sidak-corrected group differences between all groups. *and line: $p \leq 0.05$ according to a z-test of independent proportions.

Figure 3. Sign-tracking is associated with faster development of SIP, while goal-tracking predicts controlled adjunctive coping.

A) STs acquired polydipsic coping under SIP earlier than GTs. However, as SIP training progressed, GTs reached comparable levels of SIP drinking to STs. **B)** Autoshaping behaviour did not differ between rats that developed hyperdipsia (HD) and those which did not develop adjunctive coping (LD). However, goal-tracking and sign-tracking differed between rats that expressed more intermediate levels of polydipsia, whereby adaptive low intermediated drinkers (ILD) exhibited a more goal-tracker-like phenotype, making fewer CS lever presses and more CS magazine entries than both high intermediate drinkers (IHD) and compulsive HDs. Autoshaping behaviour between ILD and LD groups did not significantly differ. **C-D)** Simple

linear regression indicated that goal tracking magazine entries predicted SIP water consumption, albeit only in the ILD group. **E-F)** Sign-tracking lever presses, however, did not predict SIP water consumption in any group. For A-B), data are presented as group means ± SEM. Individual values fit with the linear regression line ± a 95% confidence interval are plotted in C-F). #p<0.05 Sidak-corrected group comparison for the indicated block. *line: p<0.05 Sidak-correct group comparison between ILD group and both IHD and HD groups. *alone: significant linear regression.

Figure 4. Acute atomoxetine administration has negligible effects on autoshaping and schedule-induced polydipsia.

A) Atomoxetine did not influence sign-tracking in any group, yet, at the high dose, it produced a trend-level reduction in goal-tracking in GTs only. **B)** The low dose of atomoxetine reduced goal-tracking behaviour selectively in ILD rats, yet this effect appeared to be driven by the high rate of CS magazine responding in these rats, as this effect did not survive an analysis of covariance controlling for baseline CS magazine entries. **C)** Acute atomoxetine did not affect well-established polydipsic responding in any group. Data are presented as group means ± SEM. **&**Sidak p<0.056 compared to vehicle. **%**Sidak p<0.05 versus vehicle, but p>0.05 with baseline CS magazine entries as a covariate.

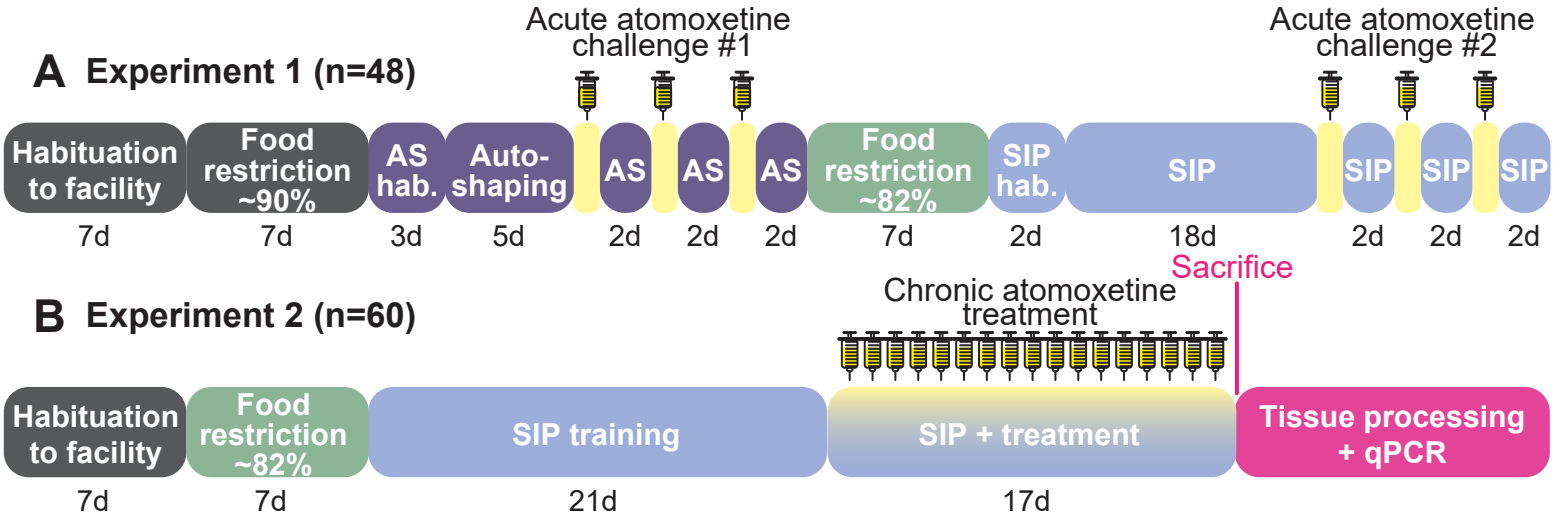
Figure 5. Chronic atomoxetine treatment selectively exacerbates well-established hyperdipsia in compulsive, high-drinking rats

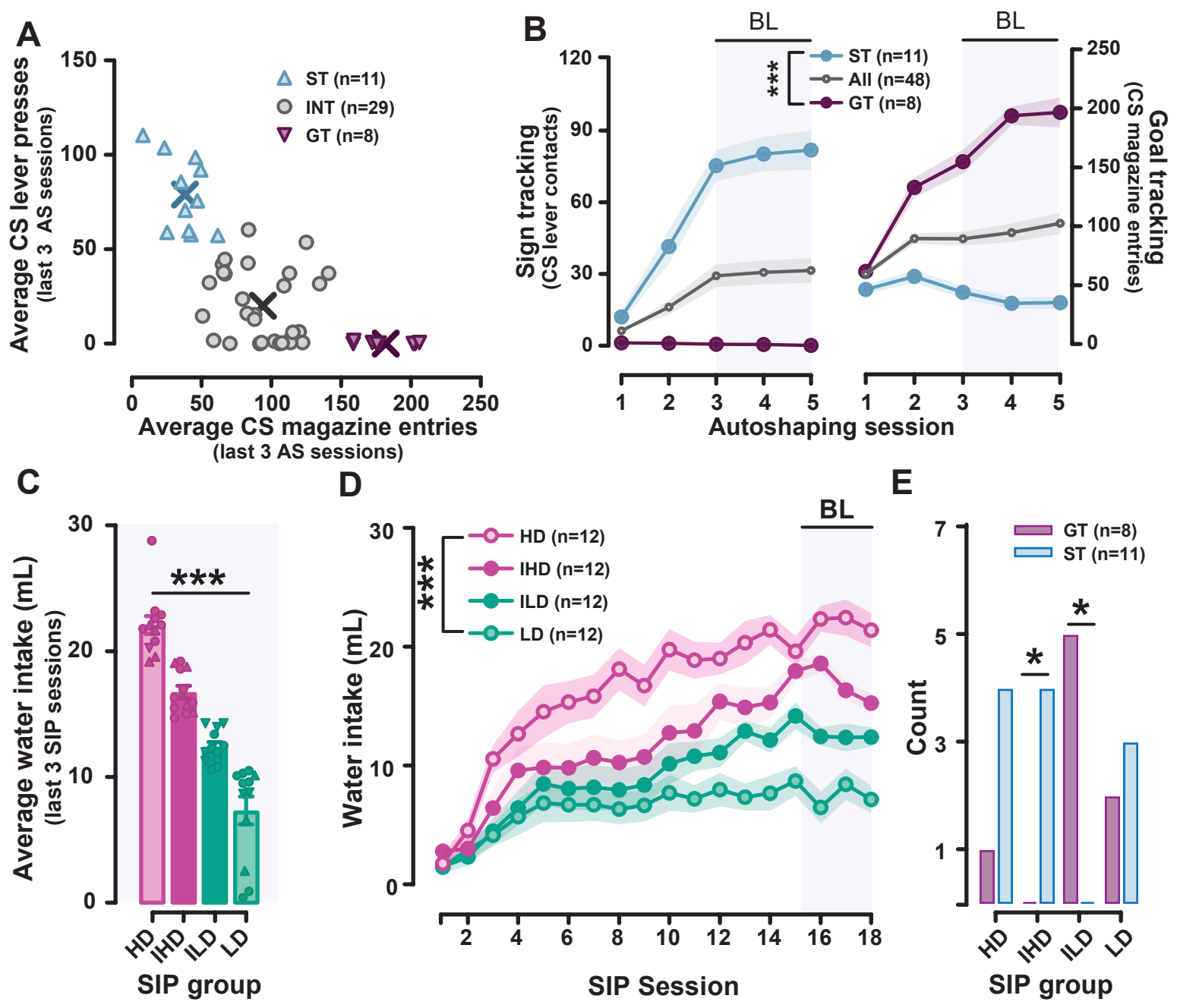
A-B) Distinct SIP phenotypes emerged across 21 days of SIP training. Groups that went on to receive chronic atomoxetine or vehicle treatment were randomly assigned and matched for baseline water consumption. **C)** Hyperdipsia displayed by HD rats was selectively exacerbated by chronic atomoxetine throughout the entirety of treatment while having no effect on drinking in any of the other groups (**C-D)**. Data are presented as group means ± SEM, with data from individual subjects shown in A and B. Blue shaded regions in A-D indicate baseline data ie. the last three drug-free sessions prior to the commencement of treatment. Treatment sessions are indicated by grey rectangles in C and D. *****p<0.05 Sidak-corrected group comparison for the indicated groups.

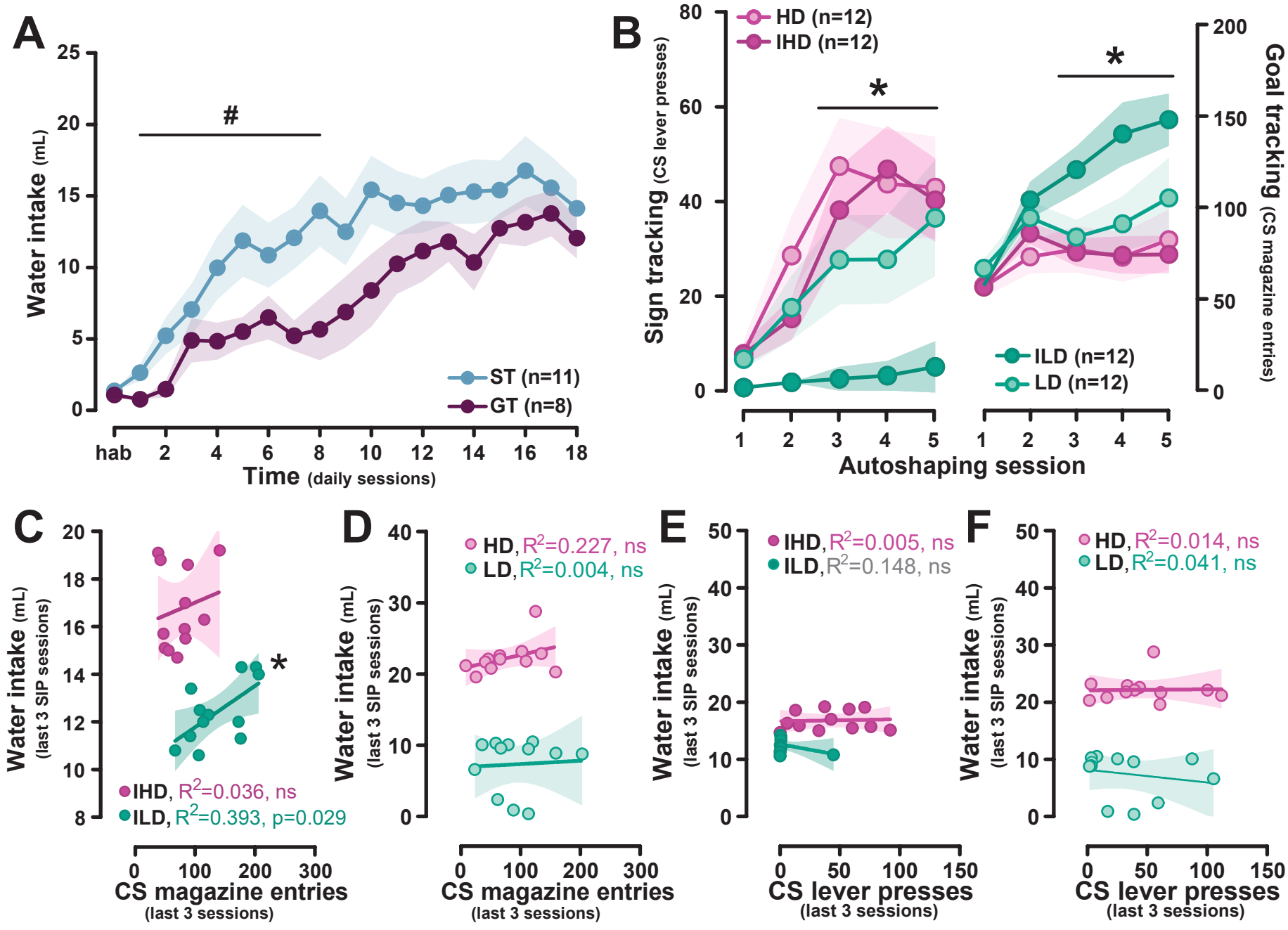
Figure 6. Chronic atomoxetine alters SIP-related cellular activity and plasticity within compulsion-relevant brain regions and results in unique transcriptional signatures within the NAcS of compulsive high-drinking and non-polydipsic low-drinking rats.

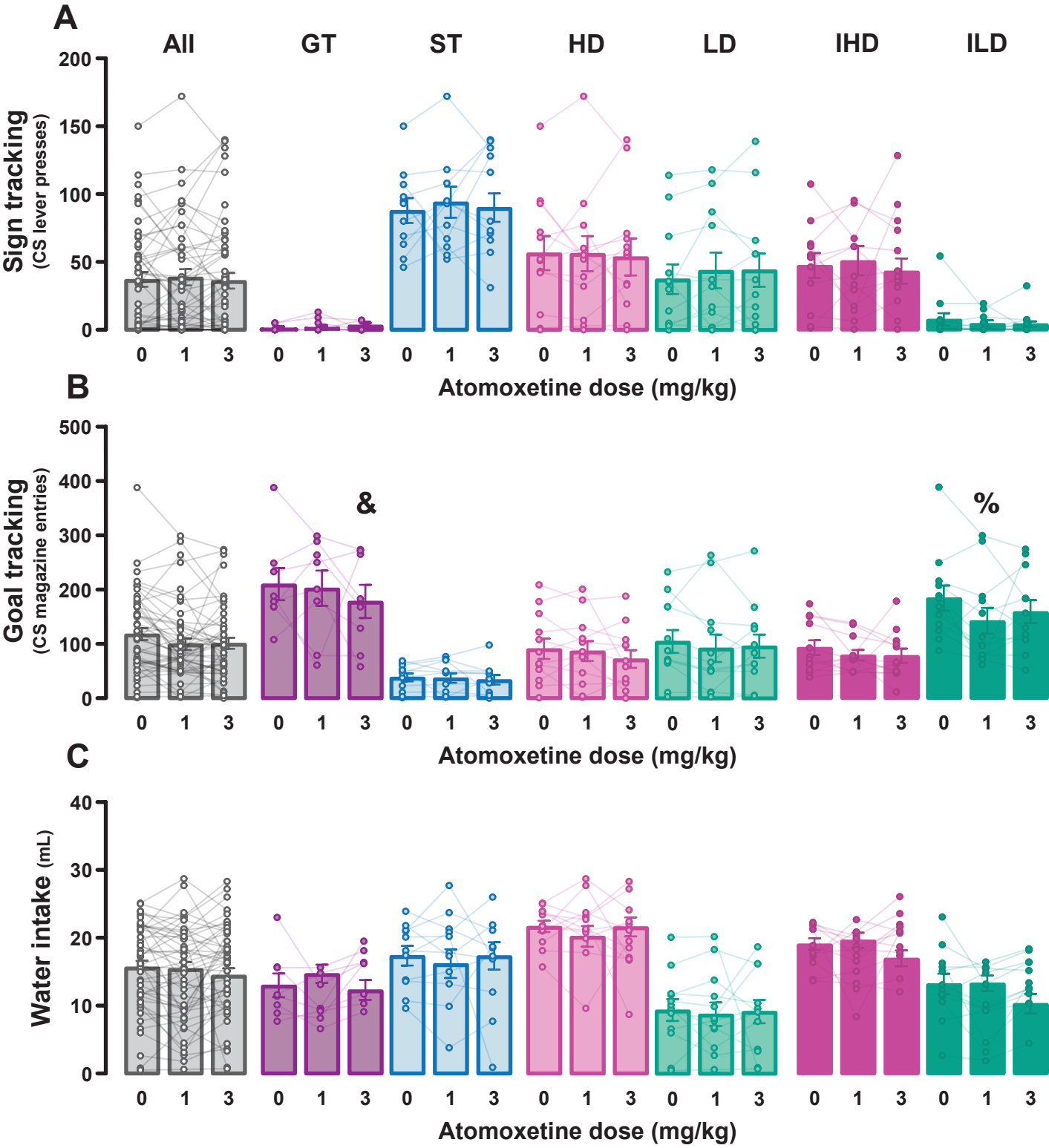
A) Compared to vehicle controls, atomoxetine-treated rats exhibited greater mRNA levels of the activity-related marker cFos in both the aDLS and pDMS, and **B)** a concomitant reduction in expression of the plasticity marker zif268 selectively in the aDLS. **C-D)** IEG levels in the insula cortex, nucleus accumbens, and amygdala were comparable between treatment groups. High and low drinkers did not differ in IEG expression across all regions of interest (see SOM). In vehicle-treated rats, zif268 expression in the **E)** aDLS and **F)** NAcC positively correlated with SIP water consumption. **G)** NAcS zif268 levels positively correlated with water drinking, yet only in vehicle-treated HD rats. Selectively in atomoxetine-treated HDs, cFos levels in the **H)** NAcC negatively related to SIP drinking, while **I)** CeA cFos levels positively correlated with water consumption. **J, K)** No significant cFos-behaviour correlations were observed in vehicle-treated HDs, nor LDs. In the NAcS, expression of IEGs and various target genes of the noradrenaline, dopamine, endogenous opioid systems were differentially correlated in **L)** vehicle-treated rats that failed to acquire polydipsia (LD) versus **M)** those which developed hyperdipsia (HD). Notably, **M)** HD controls exhibited fewer overall correlations between targe genes than **L)** vehicle-treated LDs. Chronic atomoxetine treatment also resulted in distinct transcriptional

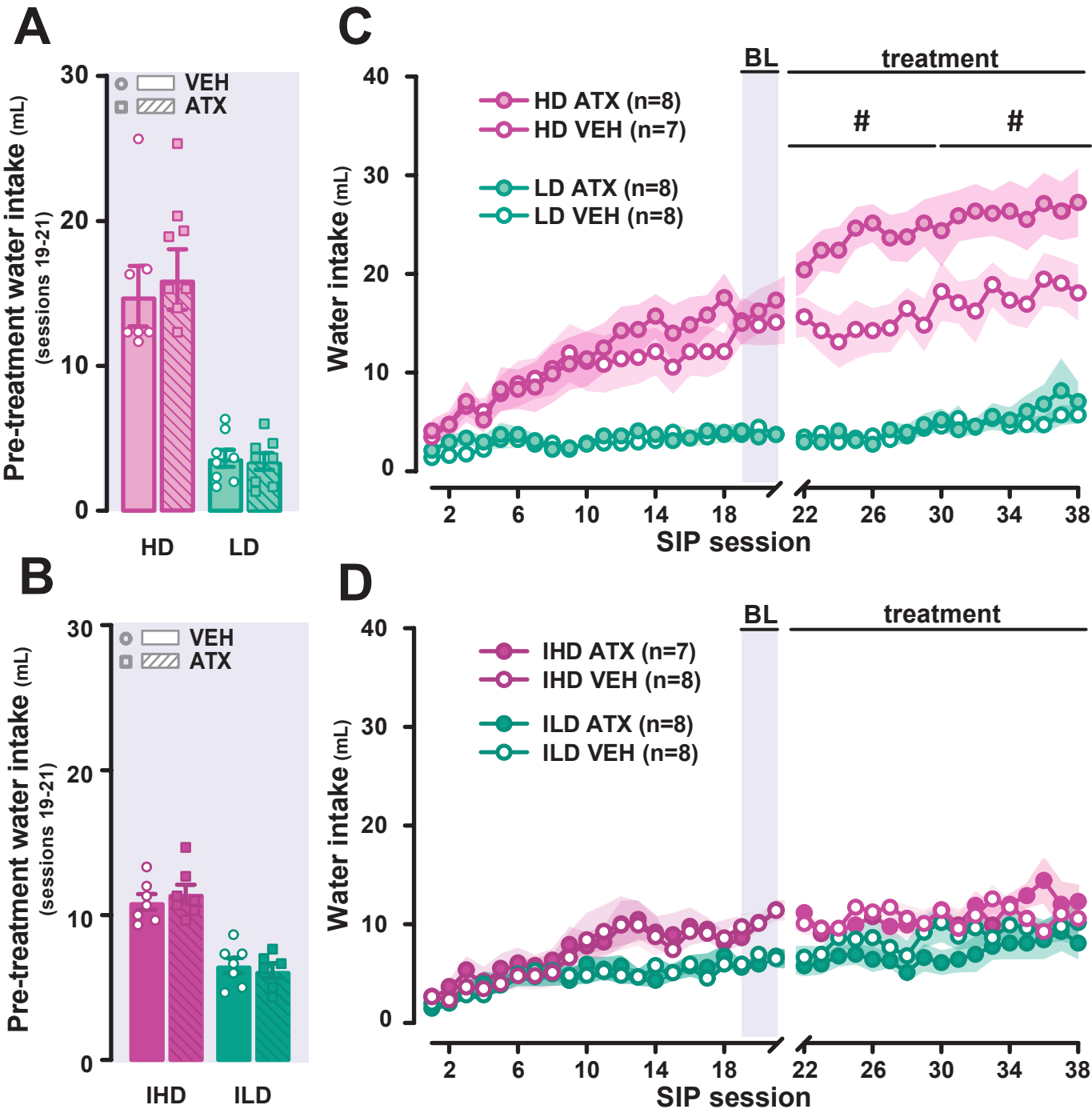
patterns in **N)** LD and **O)** HD rats. For K-N), node size represents the number of significant correlations a given gene has with other target genes, as an index of its weight in the transcriptional network. Edge width and opacity are proportional to the Spearman rho value between two nodes, and only correlations which remained significant after Benjamini-Hochberg correction are displayed. Node colour indicates the gene category: blue = noradrenaline (receptors and transporter), green = dopamine receptors, purple = kappa endogenous opioid system (peptide and receptor), yellow = IEG, orange = plasticity-related IEG. ***and line**: $p<0.05$ Sidak-corrected comparison. *** alone:** $p<0.059$ Spearman rank correlation.

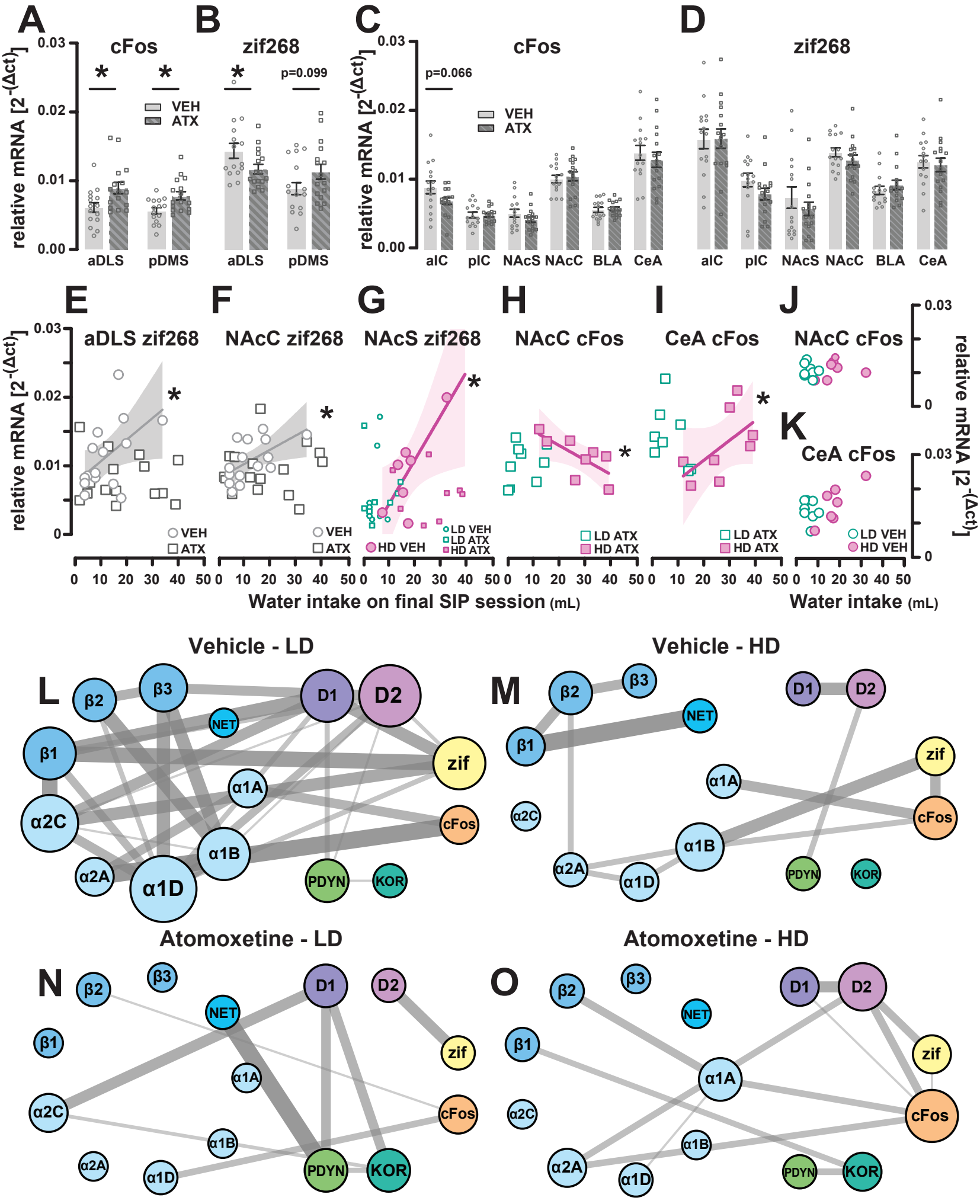

A Experiment 1 (n=48)
Acute atomoxetine challenge #1
Acute atomoxetine challenge #2
Habituation to facility
Food restriction ~90%
AS hab.
Auto-shaping
AS
AS
AS
Food restriction ~82%
SIP hab.
SIP
SIP
SIP
SIP
7d
7d
3d
5d
2d
2d
2d
7d
2d
18d
2d
2d
2d
B Experiment 2 (n=60)
Chronic atomoxetine treatment
Sacrifice
Habituation to facility
Food restriction ~82%
SIP training
SIP + treatment
Tissue processing + qPCR
7d
7d
21d
17d


A
Average CS lever presses
(last 3 AS sessions)
ST (n=11)
INT (n=29)
GT (n=8)
Average CS magazine entries
(last 3 AS sessions)
B
BL
BL
ST (n=11)
All (n=48)
GT (n=8)
***
Sign tracking
(CS lever contacts)
Goal tracking
(CS magazine entries)
Autoshaping session
C
***
Average water intake (mL)
(last 3 SIP sessions)
HD
IHD
ILD
LD
SIP group
D
BL
HD (n=12)
IHD (n=12)
ILD (n=12)
LD (n=12)
***
Water intake (mL)
SIP Session
E
GT (n=8)
ST (n=11)
*
*
Count
HD
IHD
ILD
LD
SIP group


A
Water intake (mL)
#
ST (n=11)
GT (n=8)
hab
Time (daily sessions)
B
HD (n=12)
IHD (n=12)
Sign tracking (CS lever presses)
Goal tracking (CS magazine entries)
*
*
ILD (n=12)
LD (n=12)
Autoshaping session
C
Water intake (mL) (last 3 SIP sessions)
*
IHD, R2=0.036, ns
ILD, R2=0.393, p=0.029
CS magazine entries (last 3 sessions)
D
HD, R2=0.227, ns
LD, R2=0.004, ns
Water intake (mL) (last 3 SIP sessions)
CS magazine entries (last 3 sessions)
E
IHD, R2=0.005, ns
ILD, R2=0.148, ns
Water intake (mL) (last 3 SIP sessions)
CS lever presses (last 3 sessions)
F
HD, R2=0.014, ns
LD, R2=0.041, ns
Water intake (mL) (last 3 SIP sessions)
CS lever presses (last 3 sessions)


A
All
GT
ST
HD
LD
IHD
ILD
Sign tracking
(CS lever presses)
200
150
100
50
0
0 1 3 0 1 3 0 1 3 0 1 3 0 1 3 0 1 3 0 1 3
Atomoxetine dose (mg/kg)
B
Goal tracking
(CS magazine entries)
500
400
300
200
100
0
&
%
0 1 3 0 1 3 0 1 3 0 1 3 0 1 3 0 1 3 0 1 3
Atomoxetine dose (mg/kg)
C
Water intake (mL)
40
30
20
10
0
0 1 3 0 1 3 0 1 3 0 1 3 0 1 3 0 1 3 0 1 3
Atomoxetine dose (mg/kg)


A
VEH
ATX
Pre-treatment water intake (mL)
(sessions 19-21)
30
20
10
0
HD
LD
B
VEH
ATX
Pre-treatment water intake (mL)
(sessions 19-21)
30
20
10
0
IHD
ILD
C
BL
treatment
#
#
HD ATX (n=8)
HD VEH (n=7)
LD ATX (n=8)
LD VEH (n=8)
Water intake (mL)
40
30
20
10
0
2
6
10
14
18
22
26
30
34
38
SIP session
D
BL
treatment
IHD ATX (n=7)
IHD VEH (n=8)
ILD ATX (n=8)
ILD VEH (n=8)
Water intake (mL)
40
30
20
10
0
2
6
10
14
18
22
26
30
34
38
SIP session


A
B
C
D
cFos
zif268
cFos
zif268
relative mRNA [2^-(Δct)]
0.03
0.02
0.01
0.00
VEH
ATX
*
p=0.099
p=0.066
aDLS
pDMS
aIC
pIC
NAcS
NAcC
BLA
CeA
E
F
G
H
I
J
K
aDLS zif268
NAcC zif268
NAcS zif268
NAcC cFos
CeA cFos
NAcC cFos
CeA cFos
LD VEH
LD ATX
HD VEH
HD ATX
0
10
20
30
40
50
Water intake on final SIP session (mL)
Water intake (mL)
Vehicle - LD
Vehicle - HD
Atomoxetine - LD
Atomoxetine - HD
L
M
N
O
β1
β2
β3
α1A
α1B
α1D
α2A
α2C
NET
D1
D2
zif
cFos
PDYN
KOR