Title:

Noradrenergic and Pavlovian underpinnings of compulsive versus adaptive coping strategies

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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 coping response, namely polydipsic drinking, some engage in excessive, compulsive drinking, or hyperdipsia. The development of hyperdipsia, to which highly impulsive rats are particularly vulnerable, depends on noradrenergic mechanisms, as it is prevented by the noradrenaline reuptake inhibitor, atomoxetine. However, whether noradrenergic mechanisms also underlie the maintenance of 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. Surprisingly, 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 hyperdipsia-specific 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

Stress is inevitable, and the ability to adaptively regulate and cope with negative emotions is paramount for resilience against stressors (1–4). However, some coping strategies become excessive and rigid, characteristic of psychiatric disorders such as mood/anxiety disorders (3,5,6), impulsive/compulsive spectrum disorders (ICSDs) (4,7–11), and substance use disorders (SUDs) (12–14).

Adjunctive behaviours are a form of displacement behaviour expressed by many species as a means of coping with stress (15–19). An example of adjunctive coping 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 (20,21). Polydipsic drinking is anxiolytic, as it reduces plasma corticosterone levels (21–24) and anxiety-like behaviour (25,26), which are both increased by periodic food delivery in food-restricted individuals (26–28). While the majority of rats maintain a stable, controlled level of polydipsic drinking, some lose control over their coping response and develop maladaptive, excessive SIP drinking (24–26,28–31) referred to as *hyper*dipsia. Hyperdipsia is compulsive in nature as it results in expending energy, whilst already in a caloric deficit, to consume, warm, and excrete non-regulatory volumes of water (24–26,32–34). While the acquisition of polydipsic coping depends on the mesolimbic dopaminergic system (35–37), well-established compulsive hyperdipsia is controlled by the dopamine-dependent anterior dorsolateral striatum (aDLS) habit system (25) – a shift in the neural locus of control over behaviour analogous to that which accompanies the emergence of compulsive drug-seeking habits (38–42) and OCD-like repetitive behaviours (43).

The biobehavioural factors contributing to population-level heterogeneity in coping responses and the unique vulnerability to develop compulsive manifestations of these responses in select individuals are not yet well understood. Nevertheless, impaired interoception (44,45) has been suggested to play a determinant role in the development of compulsive coping behaviours, including hyperdipsia (26,46). A greater reliance on exteroceptive contextual stimuli versus interoceptive affective and homeostatic cues to guide behaviour may facilitate the development of maladaptive coping strategies, which is supported by the role of interoceptive acuity in *resilience* to stress-related psychopathology (47–50). The anterior insula, a neural locus of interoception (51,52), is critical for the development and expression of several compulsive behaviours, including hyperdipsia (26). Interestingly, it is also involved in motor impulsivity (26), which predicts vulnerability to hyperdipsia in rats (29), and sign-tracking (53) [the propensity to attribute incentive value to, and thus approach, reward-predictive conditioned stimuli (CSs)] (54–56). Sign-trackers may also readily form Pavlovian associations with interoceptive stimuli, so that their behaviour may be more amenable to the motivational influence of internal cues (57). Accordingly, sign-trackers may more readily associate the internal state elicited by food deprivation with the anxiolytic effects of polydipsic drinking in the SIP context, a Pavlovian motivational process which would facilitate the development of this coping response.

At the neurochemical level, catecholaminergic mechanisms in the nucleus accumbens (NAc) underpin the expression of sign-tracking (58–61), motor impulsivity (62–64), and the development of adjunctive coping under a SIP procedure (35–37). Yet, the neurochemical mechanisms underlying hyperdipsia appear to be distinct from those of adaptive, controlled coping (24,29,31,65,66). The development of hyperdipsia, to which highly impulsive rats are particularly vulnerable (29,31,66), depends on noradrenergic mechanisms as it is prevented by the noradrenaline reuptake inhibitor, atomoxetine (29)- a first-line attention-deficit/hyperactivity disorder (ADHD) medication which also decreases impulsivity in hyperdipsia-vulnerable individuals (29). In contrast, atomoxetine has no effect on the development of non-compulsive,

adaptive, polydipsic drinking (29). This differential response to pharmacological manipulations of the noradrenergic system in vulnerable and resilient rats resonates with the higher extracellular noradrenaline concentration in the NAc observed in hyperdipsia-vulnerable high impulsive rats compared to their low impulsive, low drinking counterparts (66). Further, over the course of the development of hyperdipsia, compulsive rats develop abnormalities in the level of expression of the plasticity marker, Arc, in a neuronal ensemble of the noradrenergic locus coeruleus (LC) (24). These transcriptomic alterations in the LC persist long after hyperdipsia has become reliant on the aDLS dopamine (DA)-dependent habit system (25). While aberrations in the noradrenaline system confer vulnerability to develop compulsive coping, it remains unclear how noradrenergic mechanisms orchestrate the maintenance of well-established, habitual aDLS-dopamine dependent hyperdipsia. It is also unknown whether noradrenergic mechanisms mediate the potential link between NAc catecholamine-dependent behaviours, like sign-tracking, and hyperdipsia vulnerability.

Thus, in two longitudinal studies in male Sprague Dawley rats, we investigated (i) if sign-tracking predicts an increased tendency to develop hyperdipsia, and (ii) whether well-established hyperdipsia or sign-tracking is influenced by atomoxetine. Using post-mortem molecular biology assays, we characterised the influence of atomoxetine on (iii) the functional signature of hyperdipsia within the corticostriatal circuitry and (iv) compulsion-specific expression patterns of candidate genes in the NAcS, the noradrenaline-innervated (24,67,68) striatal subregion in which atomoxetine acts to ameliorate impulsivity (62).

Materials and Methods

Subjects

One hundred and eight male Sprague-Dawley rats were used in this study, as described in the **Supporting online materials (SOM)**.

Experimental design

Experiment 1 aimed to examine the relationship between sign-tracking and the propensity to develop polydipsic drinking or its compulsive manifestation, hyperdipsia, by first screening rats (n=48) for trait sign-tracking and goal-tracking in an autoshaping task (as described below), followed by training under a SIP procedure (**Figure 1A**). Experiment 1 also enabled us to assess the influence of acute manipulation of noradrenergic signaling on goal-tracking, sign-tracking, and polydipsic drinking via acute administration of atomoxetine challenge following training on both autoshaping and SIP procedures (**Figure 1A**). Experiment 2 aimed to investigate, in a separate cohort (n=60), the effect of chronic atomoxetine treatment on well-established, aDLS-dopamine habit system-dependent hyperdipsia, the same chronic treatment as previously shown to prevent the development of hyperdipsia in highly impulsive rats (25) (**Figure 1B**). Experiment 2 also enabled us to investigate the effect of atomoxetine on the neural systems and molecular signatures of hyperdipsia via post-mortem quantitative polymerase chain reaction (qPCR) analyses (**Figure 1B**).

Behavioural training

Autoshaping was conducted in twelve dedicated MEd Associates operant boxes as described previously (69,70) and detailed in the **SOM**.

SIP habituation, magazine training, and SIP training were carried out in twenty-four dedicated Med Associates boxes as described previously (29) and detailed in the **SOM**.

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 responding across the last three autoshaping sessions. Rats displaying high or low levels of adaptive coping (high intermediate drinkers, HID and low intermediate drinkers, LID, respectively), and low (low drinkers, LD) or compulsive hyperdipsic coping (high drinkers, HD) were identified based on a quartile split of adjunctive drinking performance over the last three SIP sessions.

Drugs

Atomoxetine hydrochloride (ATO, Sequoia Research Products, UK) was dissolved in sterile 0.9% saline vehicle. Atomoxetine (1.0 and/or 3.0mg/kg at 1 mL/kg, depending on the experiment) (29,71–73) and vehicle were administered intraperitoneally (i.p) 30 (29,71–73) minutes before behavioural sessions 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 thick) were µ-punched, as previously described (74) and detailed in the **SOM,** from brain regions involved in the transition from goal-directed to habitual control over behaviour (26,38,75–82): anterior and posterior insular cortex (aIC and pIC), nucleus accumbens shell and core (NAcS and NAcC), anterior dorsolateral striatum (aDLS), posterior dorsomedial striatum (pDMS), and basolateral and central amygdala (BLA and CeA). RNA was extracted and reverse transcribed into cDNA banks. Relative mRNA levels were determined in duplicate runs using qPCR as previously outlined (74) and detailed in the **SOM**. Genes of interest and respective primers are outlined 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). 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 further analysis. If sphericity was violated, the Huynh-Feldt correction was applied. 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 within-subject factor, as detailed in the **SOM**. On confirmation of significant main effects, 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. Relationships between continuous variables were assessed using Spearman correlations, and the relative distribution of subjects across SIP and autoshaping groups were compared using a Chi-squared test, as detailed in the **SOM**.

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 approach tendency (**Figure 1C**). STs made more CS-concurrent lever contacts than GTs (**Figure 1D**), which instead entered the food magazine more than the former during CS

presentation (**Figure 1D**). 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 (25,69) (**Figure 1E**). 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 (**Figure 1F**). IHD and LHD started differing from LD rats in their coping response from session 12 and 13 onwards, respectively, while differences in polydipsic drinking between them emerged after the 14th session (**Figure 1F**). GT/ST rats were unevenly distributed across SIP groups, such the IHD and ILD groups were exclusively comprised of individuals with a ST or GT trait, respectively (**Figure 1G**). There were also more GTs in the ILD group than any other SIP group (**Figure 1G**).

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) (**Figure 2A**), consistent with the relatively greater proportion of GTs in lower SIP groups (ILD, LD) (**Figure 1G**). As SIP became more established, GTs eventually reached comparable levels of coping responses to STs such that water consumption during later training sessions (d10-18) was no longer distinguishable between the groups (**Figure 2A**). We then retrospectively assessed the autoshaping profile of rats characterised by the level of polydipsia they had established in SIP. Rats that developed hyperdipsia (HD) did not differ from LD rats in goal-tracking magazine entries nor sign-tracking lever presses (**Figure 2B,C**). However, ILD rats made fewer sign-tracking lever contacts and more goal-tracking magazine entries than both IHD (**Figure 2D**) and HD rats (**Figure 2B,C&D**), 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 (**Figure 2B,C&D**).

A multivariate linear regression model was used to estimate the extent to which the level of adjunctive drinking could be predicted by both goal-tracking and sign-tracking; this did not reach significance in any SIP group (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 analyses revealed that goal-tracking magazine entries predicted the level of adjunctive drinking in the ILD group only (**Figure 2E-F**). Thus, while goal-tracking accounted for 39.3% of the variance in adjunctive drinking in ILD rats (**Figure 2E**), it did not predict their baseline, prandial drinking ($R^2 = 0.003$, $p = 0.863$), and sign-tracking alone predicted neither the level of prandial drinking ($R^2 = 0.000$, $p = 0.962$) nor that of adaptive or compulsive adjunctive drinking for any SIP group (all R^2s ≤ 0.148, ps ≥ 0.217) (**Figure 2G-H**).

Sign tracking is not influenced by acute atomoxetine treatment

Acute atomoxetine did not influence sign-tracking (**Figure 3A**) but it resulted in a slight trait-dependent decrease in goal tracking (**Figure 3B**). The highest does of atomoxetine tended to decrease magazine entries in GTs (**Figure 3B**), while the lowest dose reduced this behaviour in ILD rats (**Figure 3B**), characterised by a particularly high level of goal-tracking (**Figure 2D**). 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 (**Figure 3B**).

Acute administration of atomoxetine did not significantly affect the well-established expression of adaptive or compulsive coping behaviour (**Figure 3C**) in marked contrast with the effect of a chronic administration of the drug on either the development of hyperdipsia (see 29) or its expression, as shown below.

Experiment 2

Chronic atomoxetine treatment exacerbates compulsive coping behaviour.

As in Experiment 1, individual differences in the tendency to engage in different levels of adaptive coping or its compulsive manifestation, hyperdipsia, appeared after less than two 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 4C**), ILD, and IHD rats (**Figure 4C&D**) from session 2, 11, and 18, respectively. IHD rats demonstrated greater water drinking than LD (**Figure 4C&D**) and ILD (**Figure 4D**) rats from day 9 and 12 onward. The difference in water consumption between ILD and LD rats emerged after session 19 (**Figure 4C&D**).

After 21 SIP sessions, HD, IHD, ILD and LD rats were each randomly assigned to receive i.p. vehicle or atomoxetine (1.0mg/kg) prior to each daily SIP session for 17 days, with groups matched for baseline water consumption (**Figure 4A-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, across the entirety of treatment (**Figure 4C**), with no effect on drinking displayed by IHD and ILD rats (**Figure 4D**) or indeed LD rats (**Figure 4C**).

Molecular correlates of the exacerbation of compulsive coping by atomoxetine

Immediate early gene (IEG) hotspot analysis

Once hyperdipsia had become aDLS dopamine dependent (25), chronic treatment with atomoxetine, as compared to vehicle, resulted in all SIP-exposed rats showing increases in cFos mRNA levels in the aDLS and pDMS (**Figure 5A**), and decreases in zif268 mRNA levels in the aDLS (**Figure 5B**). These are intriguing effects, considering that the dorsal striatum does not receive noradrenergic innervation (67,83). We also observed a trend toward decreased cFos mRNA levels in the aIC ($p=0.066$) (**Figure 5C**). No differences between compulsive and non-compulsive rats and no other effects of atomoxetine were found in any other region of interest (**Figure 5C-D**).

In vehicle-treated rats, irrespective of SIP group, the level of non-regulatory water intake was associated with zif268 mRNA levels in the NAcC and the aDLS (rho = 0.524 and 0.596, respectively) (**Figure 5E, F**), while compulsive coping behaviour was specifically associated with zif268 mRNA levels in the NAcS (rho = 0.788) (**Figure 5G**). This relationship was lost in atomoxetine-treated HD rats, in which new relationships emerged between compulsive drinking and cellular activity in the NAcC and CeA that were not present in their vehicle-treated counterparts; hyperdipsia in atomoxetine-treated HD rats was negatively and positively related to cFos mRNA levels in the NAcC (rho = -0.762) and CeA (rho = 0.690, $p \leq 0.05$), respectively (**Figure 5H-I**). Neither NAcC or CeA cFos levels significantly correlated with water consumption in LDs nor in vehicle-treated rats (**Figure 5J-K**) and no other significant correlations were observed (all ps ≥ 0.073).

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

Within the NAcS, where activity-related plasticity was selectively related to compulsive coping (**Figure 5G**), the mRNA level of each of all genes of interest did not differ between vehicle- or atomoxetine-treated HD and LD rats (all Fs ≤ 2.304; all ps ≥ 0.146) (**Supplementary Figure 1A-B**). Vehicle-treated HD rats exhibited a dissipated and less interrelated transcriptional landscape than their vehicle-treated LD counterparts, as revealed by fewer overall correlations between mRNA levels for genes of interest observed in the former (**Figure 5M**) than in the latter (**Figure 5L**). Notably, in vehicle-treated HD rats, the mRNA levels of the noradrenergic receptors

(NArs) were segregated from the activity markers, cFos and zif268 (**Figure 5M**). This was in marked contrast with the high degree of convergence between these markers observed in LD rats (**Figure 5L**). In vehicle-treated LD rats, cFos and zif268 mRNA levels were closely related to those of α2A and β1 NArs, in a system where all NArs mRNA levels were highly correlated to each other. Vehicle-treated HD rats showed a very strong β1 NAr-NET mRNA correlation but no β1-Zif268 correlation. HD rats also show an emerging correlation between the mRNA levels of zif268 and cFos alongside a switch in the intensity of their respective correlation with α1B's α2A's. Chronic atomoxetine treatment resulted in unique, compulsion-specific changes in the NAcS transcriptional landscape (**Figure 5N-O**). In LD rats, it resulted in a complete disruption of the strong convergent expression of the NArs, and the loss of NArs-IEG relationships in favour of an emergent NET-kappa/dynorphin/D1 dopamine receptor (DAr) network, with D2 DAr mRNA levels being highly correlated with zif268 (**Figure 5N**). In HD rats, atomoxetine abolished the co-linearity of β NArs mRNA levels and the strong β1 NAr – NET and α1B – zif268 correlations and induced novel DArs-IEGs correlations (**Figure 5O**). Spearman rho values are presented in **Supplementary Table 2**.

Discussion

The present results reveal that sign-tracking facilitates the acquisition of adjunctive coping at the population level, while in a subgroup of rats, which adopt an adaptive coping strategy, goal-tracking positively predicts the intensity of polydipsic coping behaviour. In marked contrast to the protective effect that chronic (but not acute) atomoxetine treatment has against the *development* of hyperdipsia in vulnerable, highly impulsive rats (29), here it selectively *exacerbated* hyperdipsia once it was well-established and habitual (25). 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 contrastingly resulted in a profound reduction in plasticity in the aDLS as indicated by decreased mRNA levels of the plasticity marker zif268 (84,85). Chronic atomoxetine also altered the pattern of cellular plasticity in the incentive habit relevant NAcC-aDLS circuit (38,86), the activation of which is associated with the magnitude of adjunctive drinking, irrespective of whether it is adaptive or maladaptive. Additionally, within the striatal target of the LC, the NAcS (24,67,68), zif268 mRNA levels were uniquely related to the severity of hyperdipsia. In compulsive rats, chronic atomoxetine treatment abolished the intra-NAcS transcriptional coherence between catecholamine markers and robust β1 Nar – NET and α1B – zif268 expression correlations to favour the emergence of DArs-IEG correlations, suggesting a disengagement of noradrenergic control of behaviour in favour of dopamine.

The greater proclivity to develop adjunctive drinking shown by ST rats is consistent with an overlapping neurobiological basis of sign-tracking and SIP acquisition, both long shown to be dependent on catecholaminergic mechanisms in the NAc (35,37,59–61,87). ST and GT rats were shown here to no longer differ once SIP had become well-established and dependent on the aDLS DA-dependent habit system instead (25). Given its antecedence to and association with the negative reinforcing properties (24,25) of drinking-response-related anxiolysis (21,22,24), the SIP chamber itself may acquire incentive motivational salience as a contextual occasion setter (88). Thus, exposure to the chamber could, in and of itself, invigorate the drinking response via Pavlovian-to-instrumental transfer (PIT) (88–90). STs, which form Pavlovian associations more readily than GTs (91), may be more sensitive to this early in training. The difference between STs and GTs would be expected to disappear with further training because GTs eventually ascribe incentive motivational value to Pavlovian stimuli, similar to STs but at a slower rate (92). Goal-tracking was instead shown to be associated with the tendency to develop low levels of polydipsic drinking in intermediate rats. In these ILD rats,

goal-tracking correlated with the magnitude of the adjunctive strategy, a relationship not found in hyperdipsic, compulsive rats nor LD rats. Thus, goal-tracking may predict the predilection toward adaptive coping strategies, consistent with some theories suggesting that goal-tracking may represent an endophenotype of resilience to Substance Use Disorders (90–92, but see 69,70).

Unlike sign-tracking and well-established adjunctive drinking under SIP, goal-tracking was reduced specifically in GTs by an acute administration of the NET blocker atomoxetine. While this effect is in line with a previous report (72), the lack of effect of atomoxetine on sign-tracking may seem, at first glance, at odds with the previous study (72). However, notable 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 are defined. The previous study used a tripartite Pavlovian conditioned approach index to classify rats (72), while the present one used a k-means clustering algorithm applied to the actual sign- and goal-tracking behavioural manifestations (CS-lever contacts and CS-magazine entries). Together with a tendency for younger individuals to display higher sign tracking tendencies (96), the objective classification approach adopted in this study may result in the smaller proportion of STs observed in our experimental cohort (22% vs 40% in ref 93) and their more pronounced ST phenotype (average ~90 CS-lever contacts per session vs ~65 in ref 93). Therefore, our STs may exhibit more exaggerated Pavlovian approach tendencies that are less pervious to acute pharmacological manipulation. Acute atomoxetine treatment also had no effect on well-established SIP, thereby suggesting that chronic atomoxetine treatment, and the consequent neuroplastic adaptations, are necessary to influence habitual, aDLS DA-dependent compulsive coping behaviour.

Repeated atomoxetine treatment *exacerbated* well-established hyperdipsia in HD rats, an effect opposite to its prevention of the development of hyperdipsia in vulnerable rats (29). Together, these results suggest that the neuroplastic mechanisms engaged by protracted coping behaviour (24) are associated with alterations in noradrenergic signalling, such that noradrenaline exerts divergent control over the development of compulsive coping and its habitual, compulsive manifestation. This is in agreement with the 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 (24). Therefore, while non-vulnerable rats can engage adaptive plasticity mechanisms to maintain control of their coping strategy, HD rats may not be able to develop this strategy unless noradrenaline signalling is enhanced using atomoxetine. Once hyperdipsia is then established in HD rats, enhanced noradrenaline signalling exacerbates this compulsive coping behaviour. At the post-synaptic level, this may be associated with a switch in the transduction pathways recruited by β2 NArs from Gs to Gi (97–99) in the NAcS, identified here and elsewhere as the striatal locus of noradrenergic control of impulsivity (62) and compulsion. Further research is necessary to causally test this hypothesis. Nevertheless, the effect of atomoxetine on the development and expression of hyperdipsia demonstrates the key role played by noradrenergic mechanisms in the control of compulsive behaviour in vulnerable individuals. As development depends on the mesolimbic DA system and expression depends on the aDLS-DA habit system, this also demonstrates noradrenergic control over at least two corticostriatal circuits involved in compulsive coping behaviour.

The effect of chronic atomoxetine treatment on the compulsion-specific transcriptional landscape of the NAcS revealed by the present study sheds new light on such adaptations in the mesolimbic system. Compared to vehicle-treated LD rats, vehicle-treated HDs exhibited a profound alteration in the coherence of NArs transcriptional regulation, especially between β- and α-NArs, alongside a segregation of the NArs and DArs clusters. These alterations were accompanied by a notable narrowing of convergence between the expression of cellular activity

and plasticity markers (cFos and zif268) and NArs and DArs in compulsive individuals. Only the mRNA levels of α1A-NAr and α2A-NAr correlated with that of cFos and only α1B mRNA levels correlated with that of zif-268 in compulsive HDs. This is in marked contrast to the highly IEG-related transcription within the NAcS of vehicle-treated LD rats, whereby 5 catecholamine markers correlated with zif268 mRNA (including D1, D2-DAr, α2c- and β1-NArs) and multiple NArs correlated with cFos (α1A-Nar and α2A-Nar). Together, this indicates a privileged convergence of functional engagement of noradrenergic mechanisms in the NAcS, specifically α1A, 1B and α2A-NAr, in compulsive individuals, as opposed to a shared noradrenergic and dopaminergic contribution in LD rats. Such alterations, alongside the emergence of a coincident transcriptional relationship between the β1-NArs and the NET in compulsive rats, are generally consistent with the evidence for the independent, but interacting, role the two catecholamines play in compulsive coping (24,66,100) and compulsion vulnerability traits like impulsivity (29,66,101–103).

Interestingly, atomoxetine evokes a similar disruption 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 plasticity) of the NAcS (84,104). Furthermore, in compulsive rats, atomoxetine-exacerbated hyperdipsia was uniquely accompanied by the emergence of a relationship between the mRNA levels of DArs and those of cFos and zif268, thereby suggesting a drug-induced coupling of dopaminergic mechanisms with those mediating the functional engagement of the NAcS. One may hypothesise that this reflects a drug-induced re-engagement of the DA-dependent accumbal activity which underlies SIP acquisition (35,37,87). While it remains to be empirically tested, one clear functional consequence of such a process is an increase in the vigor of the coping response in compulsive rats, as precisely observed in atomoxetine-treated HD rats. This is predicated on the atomoxetine-induced engagement of a new ventral striatum-dependent motivational drive, on top of a well-established aDLS DA-dependent compulsive coping habit (25). This theoretical framework also leads to the prediction that atomoxetine-induced exacerbation of hyperdipsia should be accompanied by the functional engagement of the pDMS-dependent goal-directed system. Such pDMS recruitment would mediate the behavioural manifestation of the new atomoxetine-induced ventral striatum-dependent motivational process, given that the ventral striatum directs the relative engagement of goal-directed pDMS control over behaviour (86,105,106). This mechanism holds obvious similarity to compulsive relapse in individuals with an aDLS DA-dependent incentive drug-seeking habit (107). Atomoxetine treatment also blunted SIP-related plasticity, as assessed by zif268 mRNA levels (84,85), in the incentive habit system (namely NAcC-aDLS) (86) – a system in which activity-related plasticity otherwise tracks adjunctive drinking in vehicle-treated control rats. Atomoxetine contrastingly increased activity both in the aDLS and the pDMS. While neither of the dorsal striatal territories receives noradrenergic innervation (67,83), they are both under functional control of catecholaminergic mechanisms in the NAcS via spiralling striato-meso-striatal connections (38,108). In addition, aDLS DA-dependent maladaptive habits are also maintained by functional interactions with the CeA (77), which was shown here to be functionally recruited by atomoxetine in HD rats, as revealed by the emergent correlation between hyperdipsic drinking and CeA cFos mRNA levels. Together with the atomoxetine-induced reduction in plasticity in the habit system, these adaptations may lead to an exacerbation of the rigid coping habits of compulsive rats, underpinned by an inability to disengage the aDLS DA-dependent habit system – a mechanism previously shown to underlie the individual tendency to compulsively seek alcohol (109).

Overall, these data reveal a novel relationship between goal-tracking and adaptive coping, and further elucidate the differential role, and associated transcriptional signature, of ventral striatal

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 may induce or worsen compulsions in humans (110,111).

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 beahvioural experiments. ABR and MF carried out the molecular biology experiments. CC, SJ and DB analysed and interpreted the data and wrote the manuscript.

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The authors declare no competing financial interests.

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Figure legends

Figure 1. Timeline of the experiments, and characterization individual differences in autoshaping and polydipsic coping behaviour.

A) Rats in Experiment 1 were allowed 7 days of habituation after arrival at the facility, prior to being 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, habituation to the autoshaping chamber, and basic magazine training. Next, rats were trained on the autoshaping paradigm (AS) for five acquisition sessions, after which the atomoxetine challenge began according to a counterbalanced Latin square design, wherein each dosing day was followed by two days of injection-free baseline autoshaping. Following the first autoshaping-concurrent Latin square, rats were 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 same dosing and drug-free baseline schedule as for the previous Latin square. **B)** In Experiment 2, following a 1-week acclimatization period, rats were food-restricted to 80-85% of theoretical free-feeding weight. During the last two days of food restriction, rats underwent food pellet habituation, SIP box habituation and magazine training as in Experiment 1. Rats were then exposed to the SIP procedure for 21 days, during which high, high intermediate, low intermediate (ie. adaptive) and low drinkers were identified. Equal numbers of rats of 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. **C)** 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 across the last three baseline autoshaping training sessions. The “X”s represent cluster centres. **D)** Both traits were developed across the five autoshaping sessions [CS lever presses- session: $F_{4,180} = 33.161$, $p < 0.001$, $\eta^2 = 0.424$; session 1 vs 5: Sidak $p < 0.001$, $d = 1.176$; CS magazine entries- session: $F_{4,180} = 41.071$, $p < 0.001$, $\eta^2 = 0.483$; session 1 vs 5: Sidak $p < 0.001$, $d = 1.334$]. As expected, STs and GTs demonstrated greater responding either on the lever or at the food magazine, respectively, during the CS presentation [CS lever presses- session x group: $F_{8,180} = 17.240$, $p < 0.001$, $\eta^2 = 0.434$; GTs vs STs- session 1: Sidak $p = 0.003$, $d = 0.504$; all other sessions: Sidak ps < 0.001; CS magazine entries- AS session x AS group: $F_{8,180} = 23.766$, $p < 0.001$, $\eta^2 = 0.514$; GTs vs STs- session 1: Sidak $p = 0.094$; session 2: Sidak $p < 0.001$, $d = 1.165$; all other sessions: Sidak ps < 0.001]**.** **E)** Rats were defined as high (HD), high intermediate (IHD), low intermediate (ILD), or low (LD) drinkers based on a quartile split of baseline water consumption (ie. average across the last 3 days of SIP training). **F)** Adjunctive water drinking was acquired by the third day of SIP training at the population level [main effect of session: $F_{17,784} = 55.614$, $p < 0.001$, $\eta^2 = 0.558$; session 1 vs 2: Sidak $p = 0.238$, session 1 vs 3: Sidak $p < 0.001$, $d = 1.221$; session 1 vs all other sessions: all ps ≤ 0.001]. 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 baseline (BL) SIP sessions [main session x SIP group interaction: $F_{51,782} = 3.402$, $p < 0.001$, $\eta^2 = 0.188$; HD vs LD- sessions 1-2: Sidak ps ≥ 0.770; sessions 3-18: Sidak ps ≤ 0.042, ds ≥ 0.408; 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; IHD vs LD- sessions 1-11: Sidak ps ≥ 0.085; sessions 12-18: Sidak ps ≤ 0.003, ds ≥ 0.545; IHD vs ILD- sessions 1-14: Sidak ps ≥ 0.152; sessions 15-18 ps ≤ 0.037, ds ≥ 0.393; ILD v LD- sessions 1-12: Sidak ps ≥ 0.534; sessions 13-18: Sidak ps ≤ 0.054, ds ≥ 0.393]**.** **G)** Across SIP groups, there was a disproportionate

representation of GTs and STs [$\chi^2_{3,n=19}$ = 10.795, p = 0.013], such that there were fewer STs vs GTs in the ILD group [ILD- GT vs ST: z = -3.055, p = 0.002], and more STs than GTs in the IHD group [IHD- ST vs GT: z = -1.920, p = 0.055]. There were also significantly more GTs in the ILD group versus any other SIP group [STs- 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]. Data are presented as A) individual datapoints representing each subject, B-D) group means ± SEM, or E) number of subjects per group. ******* indicates significant Sidak-corrected group differences between all groups on the last three training sessions. ***** and line: p ≤ 0.05 according to a z-test of independent proportions.

Figure 2. 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 [main group x block x session interaction: $F_{8,136}$ = 2.722, p = 0.008, η^2 = 0.138; early block- SIP session x group: $F_{8,136}$ = 2.375, p = 0.020 η^2 = 0.123; and main effect of group: $F_{1,17}$ = 5.127, p = 0.037, η^2 = 0.232; 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]. However, as SIP training progressed, GTs reached comparable levels of SIP drinking to STs [main effect of group: $F_{1,17}$ = 1.830, p = 0.194 and late block SIP session x group interaction: $F_{8,136}$ = 1.330, p = 0.234]. **B-C)** Autoshaping behaviour did not differ between rats that developed hyperdipsia (HD) and those which did not develop adjunctive coping (LD) [main session x drinking group interactions for CS lever presses: $F_{12,176}$ = 2.949, p = 0.007, η^2 = 0.167 and CS magazine entries: $F_{12,176}$ = 3.968, p < 0.001, η^2 = 0.213; HD vs LD- CS lever presses: all sessions Sidak ps ≥ 0.485; CS magazine entries: all sessions Sidak ps ≥ 0.413]. However, goal-tracking and sign-tracking differed between rats that expressed more intermediate levels of polydipsia, whereby **D)** adaptive low intermediated drinkers (ILD) exhibited a more goal-tracker-like phenotype, making fewer CS lever presses [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 CS 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 high intermediate drinkers (IHD) and compulsive HD rats. Autoshaping behaviour between ILD and LD groups did not differ [ILD vs LD- CS lever presses: all sessions Sidak ps ≥ 0.146; CS magazine entries: all sessions Sidak ps ≥ 0.110]. **E-F)** Simple linear regression indicated that goal tracking magazine entries predicted SIP water consumption, albeit only 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; regression equation: average ILD water consumption = 10.032 + 0.017*(average CS magazine entries)]. **G)** Sign-tracking lever presses, however, did not predict SIP water consumption in any group [HD: $F_{1,10}$ = 0.142, p = 0.714; IHD: $F_{1,10}$ = 0.047, p = 0.832; ILD: $F_{1,10}$ = 1.733, p = 0.217; LD: $F_{1,10}$ = 0.430, p = 0.527]. For A-D), data are presented as group means ± SEM. Individual values with the linear regression line ± a 95% confidence interval are plotted in E-G). # p < 0.05 Sidak-corrected group comparison for the indicated block. * and line: p < 0.05 Sidak-corrected group comparison across the indicated sessions. * alone: significant linear regression.

Figure 3. *Acute atomoxetine administration has negligible effects on autoshaping and schedule-induced polydipsia.*

A) Atomoxetine did not influence sign-tracking in any group [CS lever presses: all Fs ≤ 0.237, ps ≥ 0.880], yet, at the high dose, it produced a trend-level reduction in goal-tracking in GTs only [CS magazine entries- dose x group: $F_{4,76}$ = 3.160, p = 0.019, η^2 = 0.143; GTs- 3.0 vs vehicle: Sidak p = 0.055, d = 0.354; all other Sidak ps ≥ 0.090]. **B)** The low dose of atomoxetine reduced goal-tracking behaviour selectively in ILD rats [main dose x SIP group interaction: $F_{6,76}$ = 2.297,

p = 0.043, η^2 = 0.154; ILD- 1.0 vs vehicle: Sidak p = 0.002, d = 0.536], 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 [main SIP x treatment interaction: $F_{6,74}$ = 2.240, p = 0.060; ILD- 1.0 vs vehicle: Sidak p = 0.372]. **C)** Acute atomoxetine did not affect well-established polydipsic responding in any group [main effect of dose: $F_{2,76}$ = 2.911, p = 0.061, η^2 = 0.071; and all dose x group interactions: Fs ≤ 2.301, ps ≥ 0.096; all rats- 1.0 vs vehicle: Sidak p = 0.998; 3.0 vs vehicle: Sidak p = 0.092]. 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.

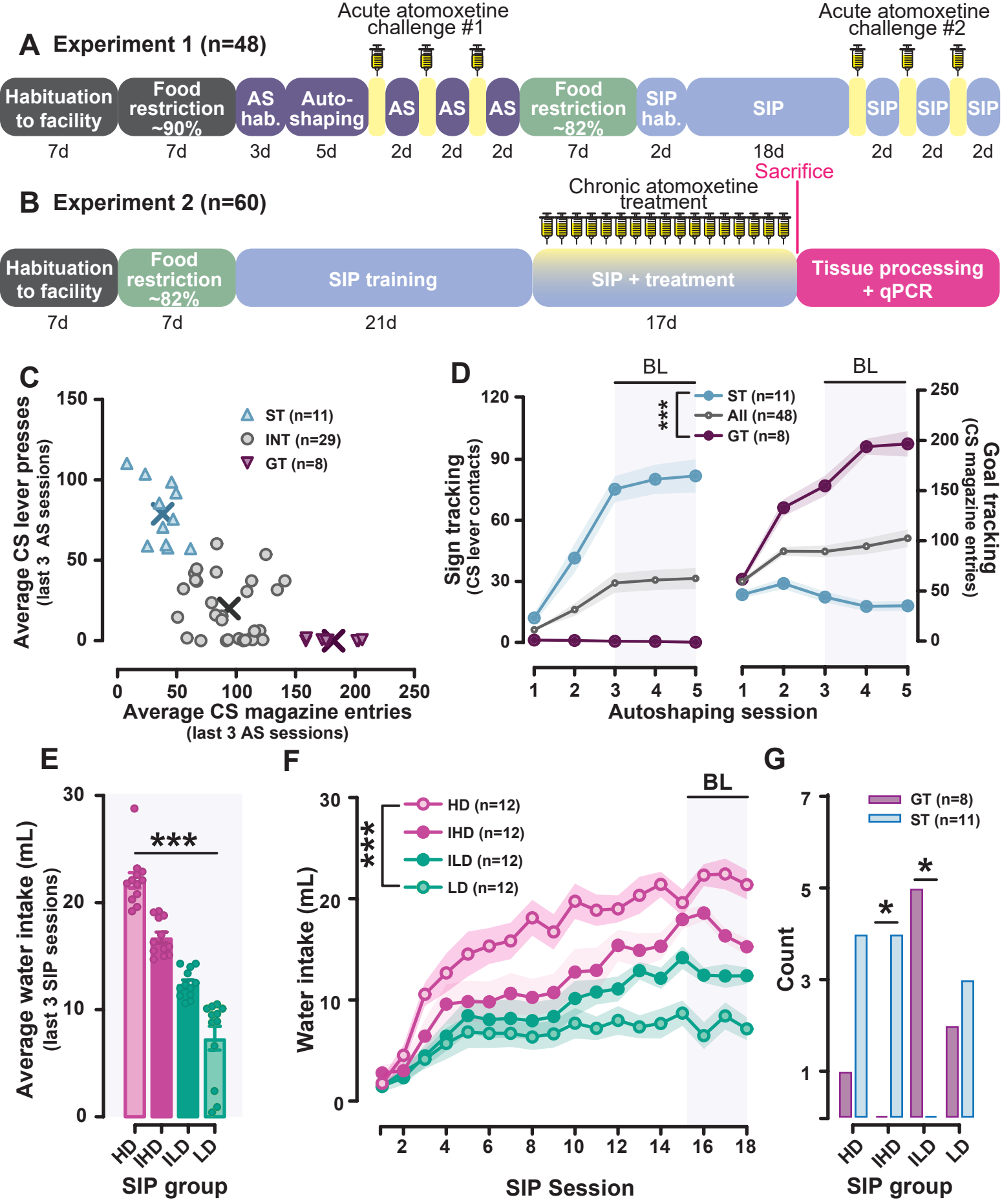
Figure 4. Chronic atomoxetine treatment selectively exacerbates well-established hyperdipsia in compulsive, high-drinking rats, an effect accompanied by differential recruitment of compulsion-relevant corticostriatal circuitry.

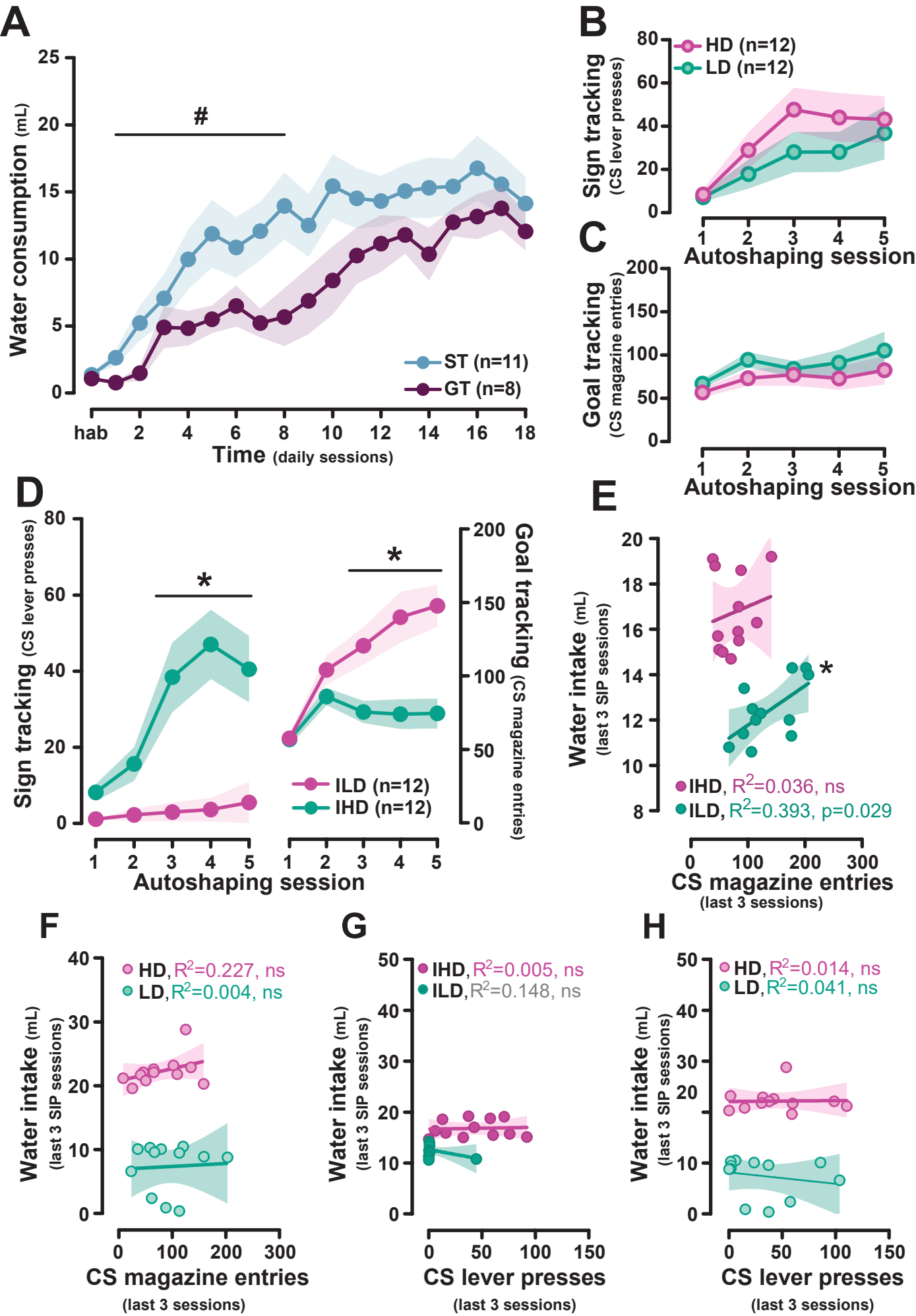
A-B) Distinct SIP phenotypes emerge across 21 days of SIP training [main session x SIP group interaction: $F_{60,980}$ = 4.952, p < 0.001, η^2 = 0.408; HD vs LD- sessions 1: p = 0.066; sessions 2-21: ps ≤ 0.054, ds ≥ 0.392; HD vs ILD- sessions 1-10: p = 0.081; sessions 11-21: ps ≤ 0.029, ds ≥ 0.380; HD vs IHD- sessions 1-17: p = 0.173; sessions 18-21: ps ≤ 0.043, ds ≥ 0.300; 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; ILD vs LD- sessions 1-19: Sidak ps ≥ 0.140; sessions 20-21: ps ≤ 0.034, ds ≥ 0.382]. Groups that went on to receive chronic atomoxetine or vehicle treatment were randomly assigned and matched for baseline water consumption [treatment group: $F_{1,50}$ = 0.432, p = 0.519; treatment group x drinking group: $F_{3,50}$ = 0.754, p = 0.525; atomoxetine vs vehicle: all groups Sidak ps ≥ 0.131]. **C)** Hyperdipsia displayed by HD rats was selectively exacerbated by chronic atomoxetine throughout the entirety of treatment [main SIP group x treatment x block interaction: $F_{9,147}$ = 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] while having no effect on drinking in any of the other groups [atomoxetine vs vehicle- early treatment block: all Sidak ps ≥ 0.117; late treatment block: all Sidak ps ≥ 0.350] (**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.

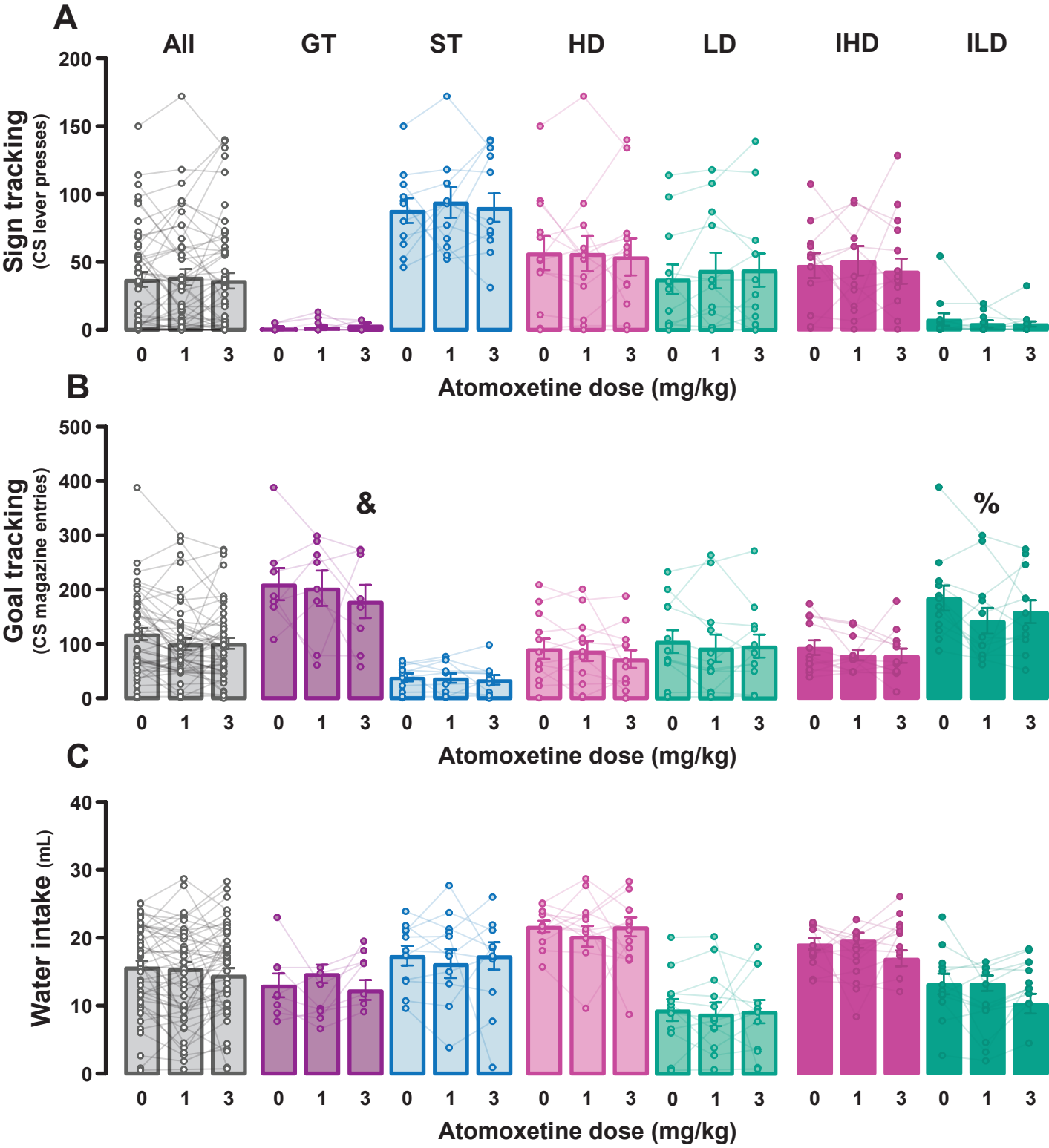
Figure 5. Chronic atomoxetine alters SIP-related cellular activity and plasticity within compulsion-relevant brain regions while differentially influencing the transcriptional NAcS landscape of compulsive, HD, and low-drinking rats.

A) Compared to vehicle controls, atomoxetine-treated rats exhibited greater mRNA levels of the activity-related marker cFos in both the aDLS [treatment: $F_{1,27}$ = 6.881, p = 0.014, η^2 = 0.203] and pDMS [treatment: $F_{1,27}$ = 4.913, p = 0.035, η^2 = 0.154], and **B)** a concomitant reduction in expression of the plasticity marker zif268 selectively in the aDLS [treatment: $F_{1,27}$ = 5.347, p = 0.029, η^2 = 0.165]. **C-D)** IEG levels in the insula cortex, nucleus accumbens, and amygdala were comparable between treatment groups [all Fs ≤ 3.668, ps ≥ 0.066]. High and low drinkers did not differ in IEG expression across all regions of interest [all Fs ≤ 1.060, ps ≥ 0.312] (data not shown). In vehicle-treated rats, zif268 expression in the **E)** aDLS [rho = 0.524, p = 0.045] and **F)** NAcC [rho = 0.596, p = 0.019] positively correlated with SIP water consumption. **G)** NAcS zif268 levels positively correlated with water drinking, yet only in vehicle-treated HD rats [rho = 0.788, p = 0.035; all other groups: all ps ≥ 0.643].Selectively in atomoxetine-treated HDs, cFos levels in the **H)** NAcC negatively related to SIP drinking [rho = -0.762, p = 0.028], while **I)** CeA cFos levels positively correlated with water consumption [rho = 0.690, p = 0.058]. **J, K)** No

significant cFos-behaviour correlations were observed in vehicle-treated HDs, nor LDs [all ps ≥ 0.094]. 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). Chronic atomoxetine treatment also resulted in divergent 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
C
Average CS lever presses (last 3 AS sessions)
Average CS magazine entries (last 3 AS sessions)
ST (n=11)
INT (n=29)
GT (n=8)
D
BL
BL
Sign tracking (CS lever contacts)
Goal tracking (CS magazine entries)
ST (n=11)
All (n=48)
GT (n=8)
***
Autoshaping session
E
Average water intake (mL) (last 3 SIP sessions)
***
HD
IHD
ILD
LD
SIP group
F
BL
Water intake (mL)
HD (n=12)
IHD (n=12)
ILD (n=12)
LD (n=12)
***
SIP Session
G
Count
GT (n=8)
ST (n=11)
*
*
HD
IHD
ILD
LD
SIP group


A
Water consumption (mL)
#
ST (n=11)
GT (n=8)
hab
Time (daily sessions)
B
Sign tracking (CS lever presses)
HD (n=12)
LD (n=12)
Autoshaping session
C
Goal tracking (CS magazine entries)
Autoshaping session
D
Sign tracking (CS lever presses)
Goal tracking (CS magazine entries)
*
*
ILD (n=12)
IHD (n=12)
Autoshaping session
E
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)
F
Water intake (mL) (last 3 SIP sessions)
HD, R2=0.227, ns
LD, R2=0.004, ns
CS magazine entries (last 3 sessions)
G
Water intake (mL) (last 3 SIP sessions)
IHD, R2=0.005, ns
ILD, R2=0.148, ns
CS lever presses (last 3 sessions)
H
Water intake (mL) (last 3 SIP sessions)
HD, R2=0.014, ns
LD, R2=0.041, ns
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)
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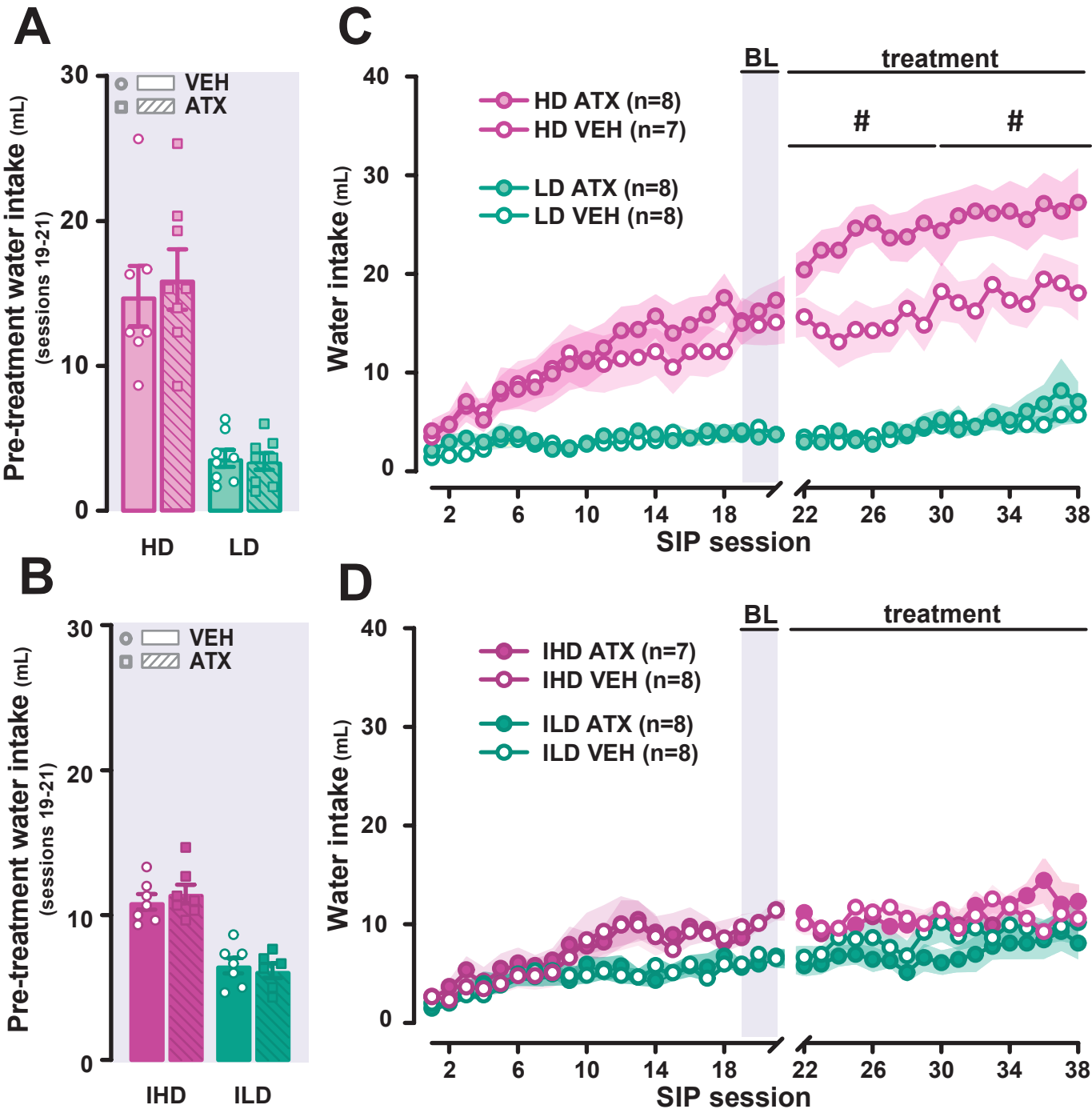
30
20
10
0
0 1 3 0 1 3 0 1 3 0 1 3 0 1 3 0 1 3 0 1 3
Atomoxetine dose (mg/kg)


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)
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30
20
10
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2
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10
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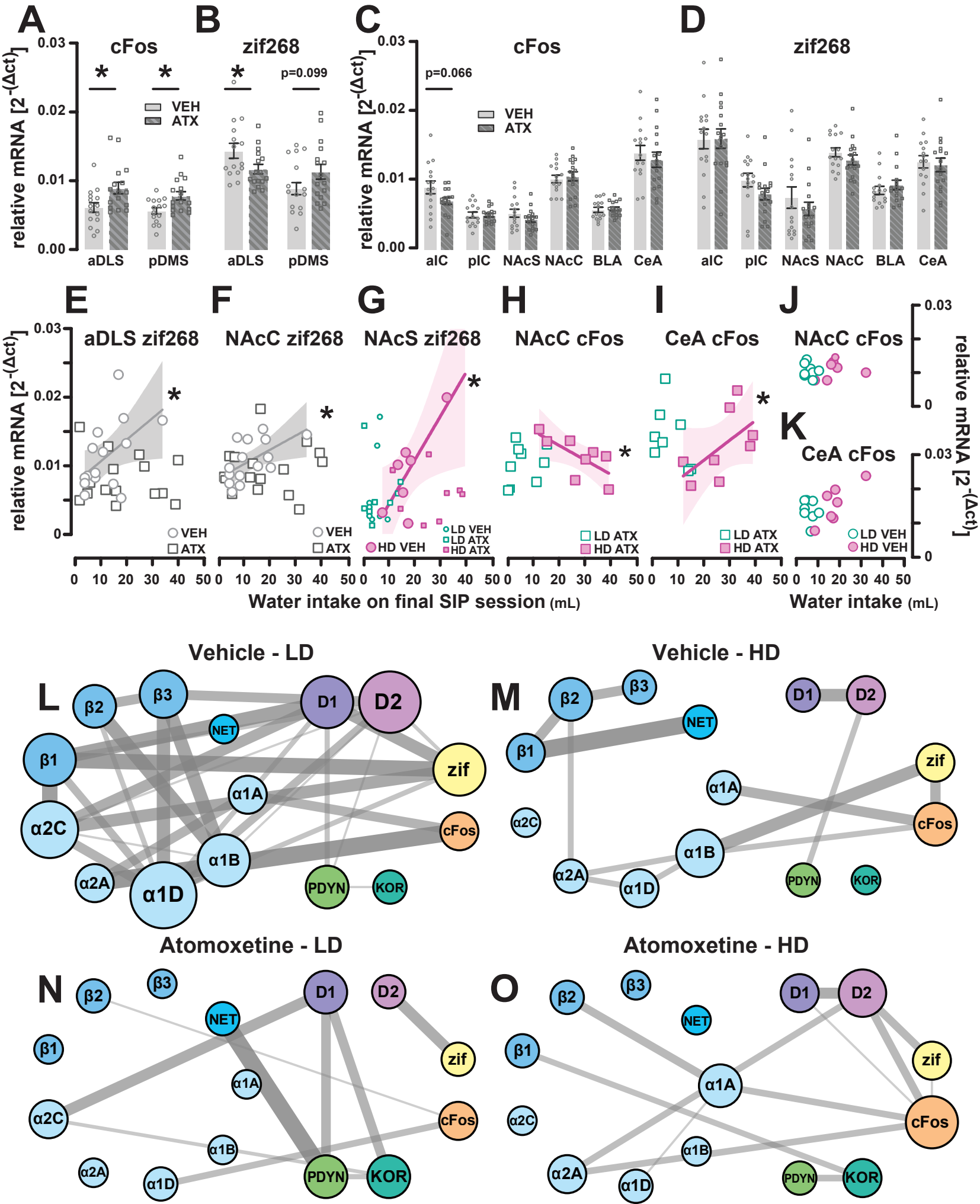
18
22
26
30
34
38
SIP session


A
B
C
D
cFos
zif268
cFos
zif268
relative mRNA [2^-(Δct)]
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0.02
0.01
0.00
*
*
*
p=0.099
p=0.066
VEH
ATX
aDLS
pDMS
aDLS
pDMS
aIC
pIC
NAcS
NAcC
BLA
CeA
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
relative mRNA [2^-(Δct)]
0.03
0.02
0.01
0
VEH
ATX
HD VEH
LD VEH
LD ATX
HD ATX
LD ATX
HD ATX
LD ATX
HD ATX
LD VEH
HD VEH
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
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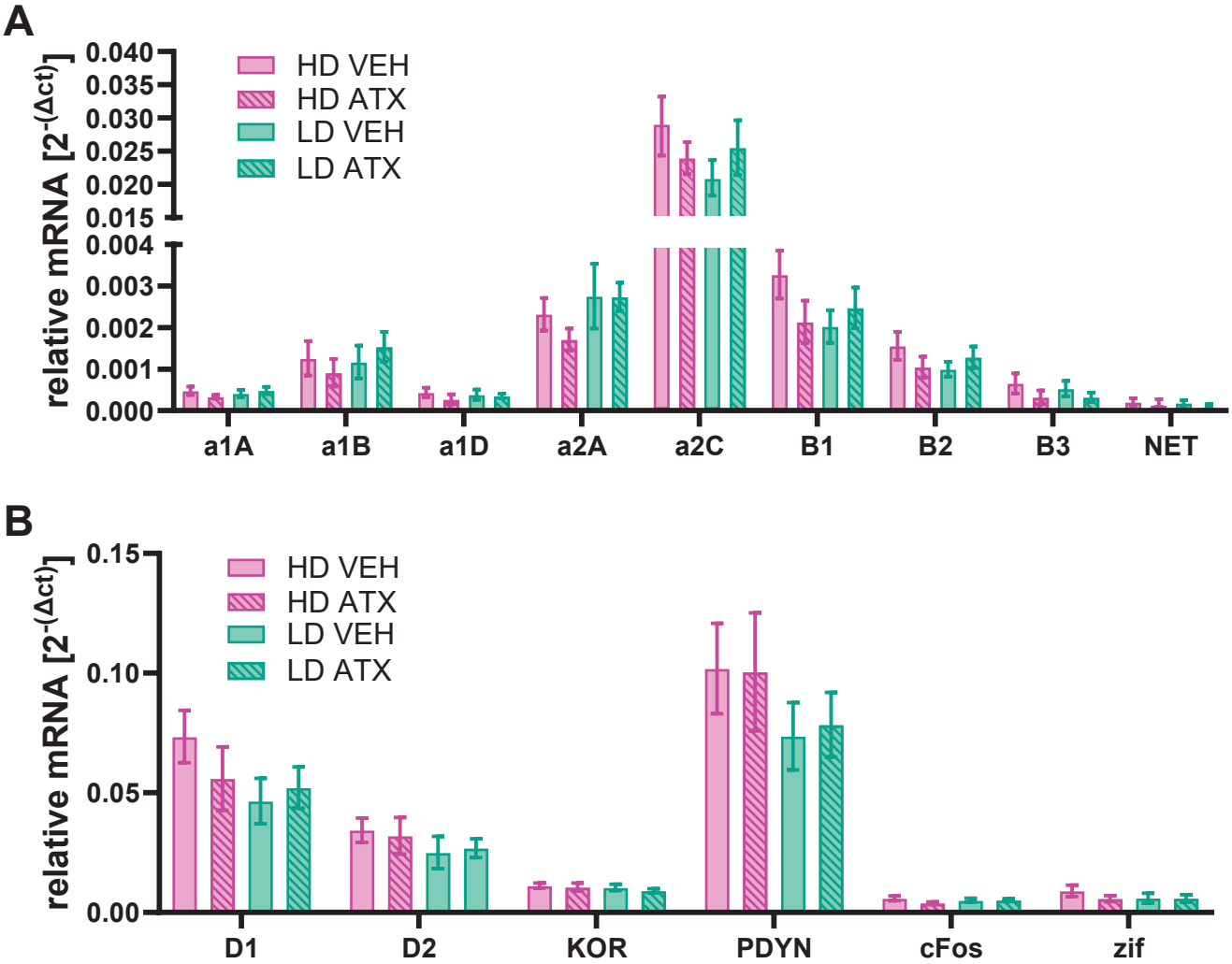
Supplemental Fig. 1