nephrolithiasis, indicate that CLC-5 and possibly other renal chloride channels, may be implicated in other disorders associated with renal stones, which account for up to 1% of all hospital admissions[2]. □

Note added in proof: Two additional genes (*CLCN6* and *CLCN7*) encoding the putative chloride channels CLC-6 and CLC-7 have also recently been cloned[28].

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Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli

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MIDBRAIN dopamine systems are crucially involved in motivational processes underlying the learning and execution of goal-directed behaviour[1–5]. Dopamine neurons in monkeys are uniformly activated by unpredicted appetitive stimuli such as food and liquid rewards and conditioned, reward-predicting stimuli. By contrast, fully predicted stimuli are ineffective[6–8], and the omission of predicted reward depresses their activity[9]. These characteristics follow associative-learning rules[10,11], suggesting that dopamine responses report an error in reward prediction[12]. Accordingly, neural network models are efficiently trained using a dopamine-like reinforcement signal[13,14]. However, it is unknown whether the responses to environmental stimuli concern specific motivational attributes or reflect more general stimulus salience[4,15]. To resolve this, we have compared dopamine impulse responses to motivationally opposing appetitive and aversive stimuli. In contrast to appetitive events, primary and conditioned non-noxious aversive stimuli either failed to activate dopamine neurons or, in cases of close resemblance with appetitive stimuli, induced weaker responses than appetitive stimuli. Thus, dopamine neurons preferentially report environmental stimuli with appetitive rather than aversive motivational value.

Two monkeys were instrumentally conditioned with visual and auditory stimuli. Appetitive conditioned stimuli eliciting lever pressing for a juice reward alternated randomly with aversive conditioned stimuli inducing hand withdrawal from a resting key in order to avoid a mild air puff to the hand or hypertonic saline to the mouth. Appetitive and aversive stimuli were adjusted to comparable motivational strength by setting juice drop size, air pressure and saline drop size slightly above a threshold below which task performance dropped sharply. Task performance was >90–95% correct. Outside the task, animals were presented with free juice drops but not with air puffs or hypertonic saline, which rapidly disrupted their collaboration.

We used two monkeys to record from dopamine neurons in midbrain catecholamine cell groups A8 (23 neurons dorsal to lateral substantia nigra), A9 (251 neurons in pars compacta of substantia nigra) and A10 (40 neurons in ventral tegmental area); we distinguished these from other midbrain neurons by their distinctive electrophysiological characteristics, which consisted of polyphasic, relatively long discharges occurring at comparatively low frequencies[6–8,16]. Most dopamine neurons were phasically activated by primary and conditioned appetitive stimuli at short mean latencies of 93–115 ms, thus confirming earlier results[6–8] (conditioned light: 100 of 128 neurons, 78%; conditioned sound: 120 of 158 neurons, 76%; free juice: 62 of 80 neurons, 78%). In contrast, very few dopamine neurons were activated by aversive stimuli, such as a conditioned sound for air puff (1 of 31 neurons, 3%), light for air puff (8 of 56 neurons, 14%) or fractal picture for saline (4 of 30 neurons, 13%) (Fig. 1). The total of 13 neurons activated by conditioned aversive stimuli belonged to groups A8 (5 of 12 neurons), A9 (7 of 89) and A10 (1 of 16), suggesting a potential mediolateral gradient for the few aversive responses. These few aversive responses failed to result in an average population response (Fig. 3*b*, *d* right). The infrequent primary aversive air puff activated only 7 of 51 neurons (14%), all of them being in A9 (Fig. 2). Sixteen of the total 20 neurons with aversive responses also showed appetitive responses. The low responsiveness to aversive stimuli cannot be attributed to movement differences which have been shown not to influence dopamine neurons[7,17,18]. Conditioned aversive stimuli elicited depressions in 36 of 117 neurons (31%; Fig. 1, bottom), reminiscent of depressions related to the omission of expected reward[9].

The chronology of experiments demonstrates the way dopamine neurons were preferentially activated by appetitive stimuli. The first animal was extensively conditioned with a small light for appetitive outcome. Later, an adjacent, similar light of a different colour served as the aversive stimulus associated with air-puff avoidance and alternated randomly with the appetitive light, the animal discriminating well between them. Dopamine neurons discriminated quantitatively between these motivationally opposing stimuli, the appetitive light activating the majority of them whereas the similar aversive light activated fewer neurons and at lower magnitudes (Fig. 3*a*). In order to distinguish between genuinely, albeit lower, aversive responsiveness and confounding generalization to appetitive stimuli, we subsequently conditioned an auditory aversive stimulus previously not associated with appetitive outcome. As already described, only 1 of 31 neurons was activated by the aversive sound (Fig. 3*b*), suggesting that the aversive activations of Fig. 3*a* were due to stimulus generalization. In a third step, we tested the general effectiveness of auditory stimuli by reversing stimulus modalities and indeed found most neurons to be activated by the appetitive sound (Fig. 3*c*). Although behavioural response to the appetitive light had been extinguished, the similar aversive light was still effective, albeit with many fewer neurons and at lower response magnitude. This responsiveness may either suggest a remaining appetitive 'tag' on visual stimuli or a general visual response preference. When testing this, we avoided stimulus generalization by presenting

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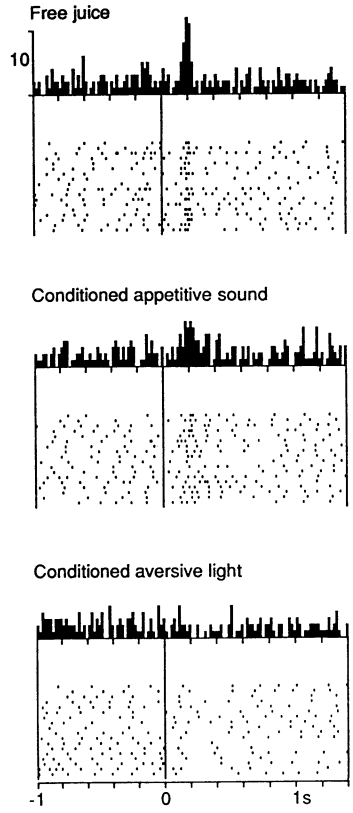

FIG. 1 Selectivity of activations of one dopamine neuron by primary and conditioned appetitive stimuli. Neuronal activity increased after a drop of free juice outside any task (top) and after a conditioned sound eliciting a reaching movement for juice reward (middle). Activations were absent after a conditioned light in air-puff avoidance trials (bottom). However, a small depression occurred after the stimulus. Histograms are composed of neuronal impulses shown as dots below. Each dot denotes the time of a neuronal impulse, their distances to stimuli representing real time intervals. Each line of dots shows one trial, the original sequence being from top to bottom. The neuron was recorded in group A9 (substantia nigra). Vertical calibration in 10 impulses per bin applies to all histograms. METHODS. All neurons were tested in randomly alternating appetitive and aversive trials. Free-juice trials outside any task were given in separate blocks. In appetitive trials, the animal released a resting key in reaction to a light or sound stimulus and touched a small lever in front at 20 mm below eye level and 27° lateral in order to receive 0.15 ml of apple juice through a spout at the mouth. Liquid arrived at the mouth 55 ms after the electronic pulse activated the liquid valve. In aversive trials, the animal withdrew its hand from the resting key within 1.5 s after a visual or sound stimulus in order to avoid a mild air puff (2–4 bar) to the hand or delivery of 0.15 ml of 1.7 M NaCl through another spout to the mouth (active avoidance task). Conditioned stimuli were (1) a square yellow or red light-emitting diode (11 × 11 mm) 40 mm above the lever; (2) a green, round light-emitting diode (3 mm diameter) 10 mm above the lever; (3) a 100-ms-long 1 kHz sound 10 mm above the lever; and (4) a coloured fractal image (13° × 13°) on a video screen 260 mm from the animal's eyes. In free-juice trials, animals received the same amount of apple juice at irregular intervals. Recording sites of dopamine neurons randomly sampled from cell groups A8, A9 and A10 were marked with small electrolytic lesions and reconstructed from 40-μm-thick, tyrosine-hydroxylase-immunoreacted or cresyl-violet-stained coronal brain sections. As previously described[6–8,16], single dopamine neurons recorded extracellularly discharged polyphasic, initially negative or positive impulses with relatively long durations (1.8–5.5 ms) and low frequencies (0.5–8.0 imp s^{-1}). Impulses contrasted with those of pars reticulata neurons of substantia nigra (70–90 imp s^{-1} and <1.1 ms duration) and neighbouring fibres (< 0.4 ms duration). Neuronal activations were compared against a 500-ms prestimulus control period by using a Wilcoxon procedure with constant time windows comprising 80% of onset and offset times of statistically significant increases ($P < 0.01$; refs 6–8) (86–325 ms after sound, 76–215 ms after light, 126–270 ms after liquid). Neuronal responsiveness was assessed in terms of (1) numbers of neurons with significant changes in the time window and (2) magnitude of activation in the time window for every neuron tested, independent of a significant response. All experimental protocols conformed to the Swiss Animal Protection Law and were supervised by the Fribourg Cantonal Veterinary Office.

aversive visual stimuli to the second animal before it had ever been conditioned with appetitive visual stimuli. In step four, very few neurons were activated by an aversive light associated with air-puff avoidance (Fig. 3*d*) and, in step five, by a picture associated with saline avoidance (Fig. 3*e*), thus arguing against a visual response preference.

Thus, dopamine activations report the appetitive value of primary and conditioned environmental stimuli. Activations hardly occur after non-noxious aversive stimuli in most situations. Only when aversive stimuli appear in close temporal proximity and in random alternation with physically very similar appetitive stimuli, the discrimination loses its all-or-none character and is expressed by a quantitative preference for appetitive stimuli. The activations are broadcast simultaneously through widespread projections to the striatum and frontal cortex. They are particularly effective for increasing dopamine concentration[19] at specific synapses[20,21]. Apparently complementary functions are carried out by dopamine release detected by voltammetry and dialysis methods, which occurs with a lower time course and in a larger behavioural spectrum including aversive events[22–25]. Such release appears to be largely due to synaptic overflow[26] and possibly extrasynaptic release, and would predominantly affect extrasynaptic receptors[27].

The responses to appetitive stimuli and the occasional generalization to physically similar but aversive stimuli apparently reflect an appetitive 'tag' that has been affixed to stimuli through the previous experience of the animal. Stimulus generalization even occurred to a small extent with stimuli whose appetitive associations were behaviourally extinguished (Fig. 3*c*). Thus dopamine neurons maintain central representations of a maximum range of stimuli that are appetitive or resemble current or previous appetitive stimuli. The responses could be useful in behaviour for exploring new, potentially appetitive stimuli with similar physical features or for testing extinguished appetitive stimuli.

The quick and temporally precise dopamine response to appetitive stimuli may serve two functions. Firstly, the quick activation can be used for the early initiation process of rapid approach reactions. Slower systems with more time for stimulus evaluation could process further stimulus details and provide decisive supplementary information for finalizing behavioural

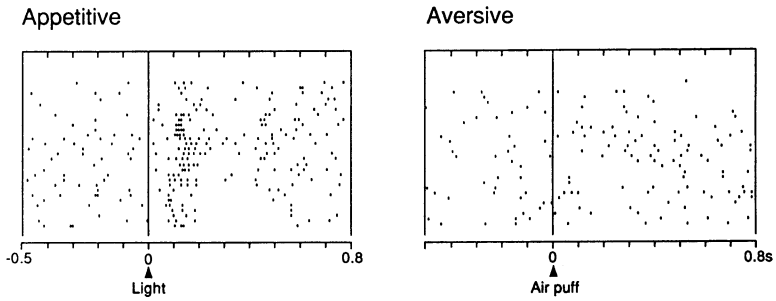

FIG. 2 Lack of response of nine dopamine neurons to aversive air puff (right). Most of these neurons were activated by a conditioned appetitive light eliciting a reaching movement for liquid reward(left). Air puffs occurred when the animal failed to release the resting key within 1.5 s after a conditioned auditory stimulus. Rasters to the right display neuronal activity from all trials with air puffs (2–5 trials per neuron), the left showing matching numbers of randomly interspersed appetitive trials from the same neurons at corresponding vertical levels of display.

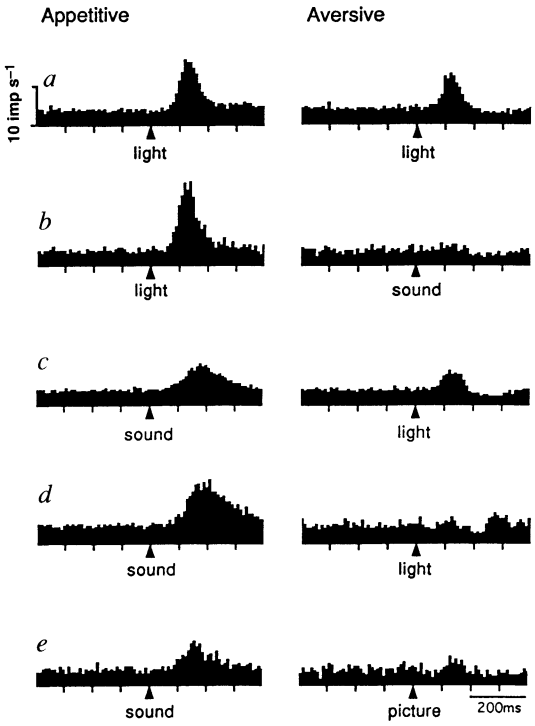

FIG. 3 Chronology of experimentation from *a* to *e* shows preferential or exclusive activation of dopamine neurons by appetitive stimuli. Population histograms show neuronal data from the five combinations of randomly alternating appetitive and aversive trials. Data are separated for analysis according to trial type. *a*, Activations in the first monkey elicited by two similar lights serving as appetitive and aversive conditioned stimuli in randomly alternating trials. Of 97 neurons tested, 73 (75%) were activated by an appetitive round green light (mean magnitude, 197% above control) and 63 (65%) by an aversive square yellow light (magnitude 162%; $P < 0.03$; paired *t*-test). *b*, Subsequent separation of sensory modalities revealed an exclusive appetitive activation, thus explaining the aversive activations in *a* with appetitive stimulus generalization. Of 31 neurons, 27 (87%) were activated by the appetitive round green light, but only 1 by the aversive sound. *c*, Subsequent reversal of modalities demonstrated effectiveness of sounds for activating dopamine neurons. Of 72 neurons, 54 (75%) were activated by the appetitive sound (magnitude, 160%), and 23 (32%) by the aversive square red light (magnitude, 100%; $P > 0.01$). *d*, Lack of activation by an aversive square red light in the second monkey which was inexperienced with experimental visual appetitive stimuli. Of 56 neurons, 47 (84%) were activated by the appetitive sound, but only 8 (14%) by the aversive light. Thus, visual aversive activations in *a* and *c* were apparently due to stimulus generalization. *e*, Lack of activation by a coloured fractal picture conditioned as an aversive stimulus in the second monkey. A drop of hypertonic saline served as the primary aversive stimulus, instead of the air puff in *a*–*d*. Of 30 neurons, 19 (63%) were activated by the appetitive sound, but only 4 (13%) by the aversive picture. The results of *d* and *e* demonstrate the ineffectiveness of aversive stimuli across a considerable range. For each display, histograms from each neuron tested were normalized for trial number, added together, and the resulting sum was divided by the number of neurons. Dopamine neurons were randomly sampled in two monkeys from cell groups A8, A9 and A10, throughout steps *a*–*e*.

reactions. A candidate is the amygdala with its involvement in reward associations[28,29] and its highly differentiated responses of longer latencies to reward-associated stimuli[30]. Secondly, as a temporally precise, global reinforcement signal, the response can exert a temporal selective effect on those synaptic processes that are specifically engaged in the behaviour to be reinforced, thus leading to dopamine-mediated long term changes suggested by the correspondence with learning rules[12–14]. □

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Calcium-dependent interaction of N-type calcium channels with the synaptic core complex

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NEUROTRANSMITTER release is initiated by influx of Ca^{2+} through voltage-gated Ca^{2+} channels[1,2], within 200 μs of the action potential arriving at the synaptic terminal[3], as the Ca^{2+} concentration increases from 100 nM to >200 μM[4]. Exocytosis requires high Ca^{2+} concentration, with a threshold of 20–50 μM and half-maximal activation at 190 μM[5,6]. The synaptic membrane proteins syntaxin[7,8], 25K synaptosome-associated protein (SNAP25)[9], and vesicle-associated membrane protein (VAMP)/synaptobrevin[10–12], are thought to form a synaptic core complex which mediates vesicle docking and membrane fusion[13–19]. Synaptotagmin may be the low-affinity Ca^{2+}-sensor[20–24], but other Ca^{2+}-sensors are involved[25–27] as residual neurotransmission persists in synaptotagmin-null mutants. Syntaxin binds to N-type Ca^{2+} channels[7,8,28,29] at a site in the intracellular loop connecting domains II and III[30]. Here we describe Ca^{2+}-dependent interaction of this site with syntaxin and SNAP25 which has a biphasic dependence on Ca^{2+}, with maximal binding at 20 μM free Ca^{2+}, near the threshold for transmitter release. Ca^{2+}-dependent interaction of Ca^{2+} channels with the synaptic core complex may be important for Ca^{2+}-dependent docking and fusion of synaptic vesicles.

To analyse the Ca^{2+} dependence of association of the cytoplasmic loop connecting domains II and III (L_{II-III}) of the α_{1B}-subunit of N-type Ca^{2+} channels with syntaxin 1A, we measured the binding of two fusion proteins tagged with epitopes containing six histidines (L_{II-III}(718–963) and L_{II-III}(773–859)) to recombinant

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